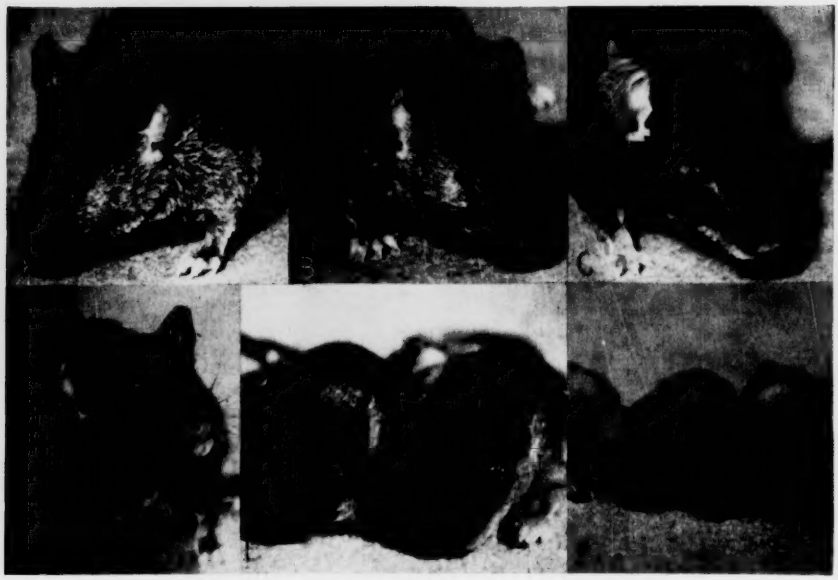
The Journal of HEREDITY



SQUINT CATARACT—A NEW MUTATION

(SEE PAGE 13)

Mutant Maize

Mammalian Chromosomes in Vitro

Academician Lysenko on Species

Pattern of Tetraploidy in the Apple

VOLUME 44 JANUARY-FEBRUARY, 1953 NUMBER 1

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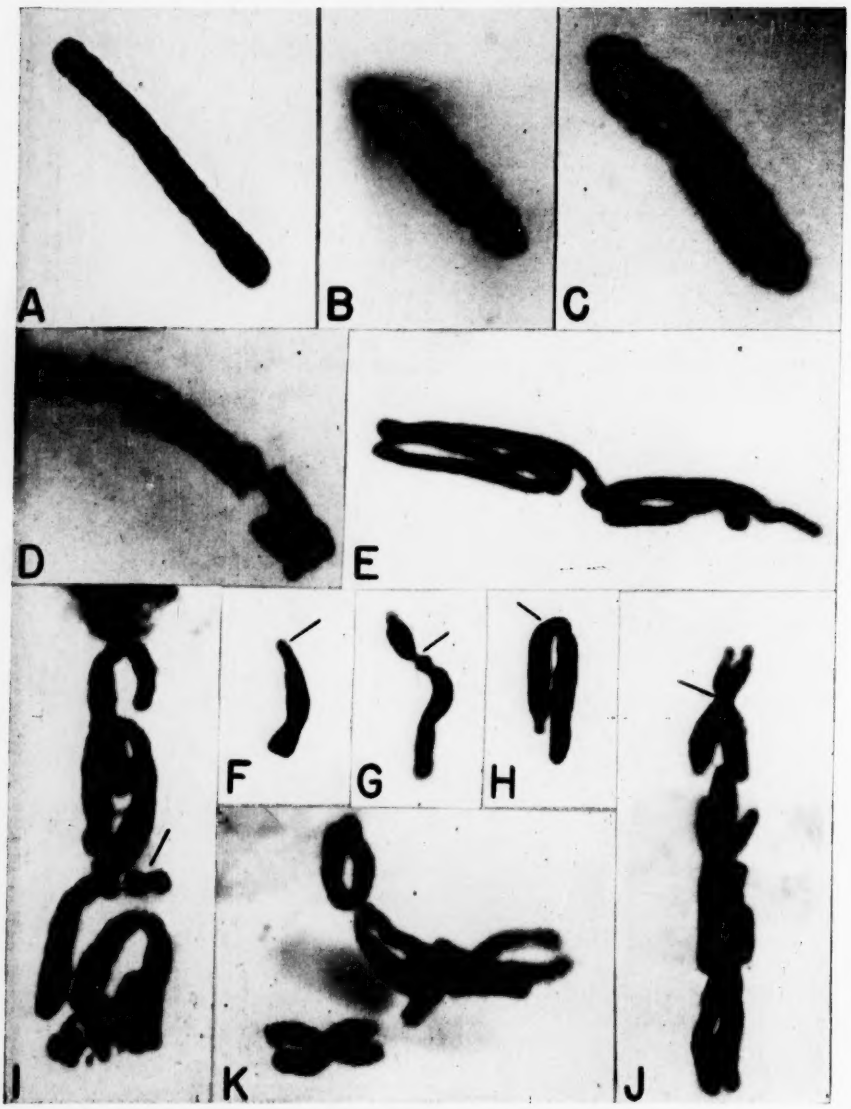

POLLEN TUBE MITOSIS AT DIFFERENT TEMPERATURES

Figure 1

A—Generative nucleus in interphase. *B*, *C* and *D*—Prophase: beginning of early, mid and late prophase, respectively. *E*—Metaphase at room temperature—chromosomes long and thin. *F-K*—Metaphase chromosomes at low temperature (*F* at 3°C., *G-K* at 6°C.); kinetochores indicated by lines. *I*—the A chromosome with three separate differentiated segments and *F*—two such segments partially "fused." *G*—the most common pattern in the B chromosome, the short arm with a terminal and the long arm with an interstitial region differentiated. *J*—the C chromosome with chromatids widely separated and the end of the short arm differentiated. *H*—the typical pattern of the D chromosome, the short arm with one terminal, the long arm with one interstitial segment differentiated. *K*—an unusual colchicine-type effect occasionally seen after cold treatment. Magnification 1275×.

THE REACTION OF TRILLIUM POLLEN-TUBE CHROMOSOMES

To Cold Treatment During Mitosis

E. R. BOOTHROYD*

LOW temperatures have been used by many workers as an aid to the study of chromosome structure. The usual effect of cold is to cause supercontraction of the chromosomes, with consequent straightening. This frequently makes the kinetochore region and other constrictions more readily distinguishable than they would be at normal room temperatures. In the chromosomes of certain plants and animals, segments have been found which respond to cold in a striking manner. These segments, although morphologically similar to the other parts of the chromosomes when mitosis occurs at room temperature, are readily distinguishable at low temperatures. From late prophase to early telophase, under the latter conditions, they have less affinity for nuclear stains (including Feulgen's reaction) and usually have a smaller diameter than the rest of the chromosomes. This "differential reaction" of various parts of chromosomes to low temperatures has been most widely studied in the genus *Trillium* (Bailey[1]; Darlington and La Cour[6, 7]; Haga[9]; Haga and Kurabayashi[10, 11]; La Cour[14]; Resende, Lemos-Pereira and Cabral[15]; Resende, Salord and Leite-Rio[16]; Wilson and Boothroyd[19, 20]). The segments which show the special response to cold have been variously described as genetically inert heterochromatin which had been starved of nucleic acid during interphase (Darlington and La Cour[6, 7]), as nucleolar zones (Resende *et al.*[15]) and as regions which were less contracted than the other parts of the chromosomes (Wilson and Boothroyd[20]).

Most studies of "differential reactivity," with the exception of Callan's[4] work on meiosis in *Triton* and Darlington and La Cour's[7] report of differentiation in the pollen grain mitosis of *Fritillaria*, have been in somatic tissues (e.g. root tips) where the mitotic cycle is unknown. It was felt that this left a very serious gap in the information on which any theory concerning differential reactivity could be based. The present investigation was begun, therefore, primarily in the hope of obtaining direct evidence on the relationship between the time in the mitotic cycle at which cold treatment was initiated and the differential response within the chromosomes. Pollen-tube divisions were used because here the division cycle (a single mitosis) is known and unlike the pollen-grain division the rate of mitosis is rapid enough for convenient study. Preliminary results (Boothroyd[2]) indicated that treatment during prophase could induce a differential reaction in the chromosomes.

Material and Methods

Plants of *Trillium erectum* L. were collected in the vicinity of Montreal, Quebec, and kept at room temperature for several weeks. Mature pollen grains were germinated at room temperature in hanging drops of 3 percent sucrose solution. After various lengths of time cultures were transferred to the refrigerator at 3° or 6°C. On removal from the refrigerator the material was immediately fixed and stained in aceto-carmine and mounted in a Venetian turpentine medium (Wilson[18]).

Measurements were made from camera lucida drawings at a magnification of 4500×, for which a Zeiss 120×, 1.30 N.A. objective and 20× ocular were used.

Observations and Discussion

Preliminary studies. In earlier work on *Trillium* root tips it was found that a temperature of 3°C. was satisfactory for the induction

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of a differential reaction in the metaphase chromosomes (cf. Wilson and Boothroyd[20]). However, when pollen tubes which had been cultured at room temperature were transferred to 3°C. mitosis was virtually stopped, nuclei became abnormal and many tubes collapsed. That mitosis is arrested at 3°C. may be seen from Table I, which shows the frequencies of various stages of mitosis after different treatments. Even after 212 hours at 3°C., following 20-21 hours at room temperature, the distribution of mitotic stages was the same as in the controls fixed after the initial culturing at room temperature. When 6°C. was used for the cold treatment mitosis progressed slowly, as may be seen by comparing cold-treated materials with their controls in Table I. Consequently this was the temperature used for most of the cold treatments reported here.

Pollen-tube cultures were kept at room temperature for times ranging from 20 to 43 hours and then refrigerated at 6°C. for from 92 to 173 hours. After 20-21 hours at room temperature 25 percent of the generative nuclei were still in interkinesis and the remaining 75 percent in prophase, mostly early (the classification of prophases as early, mid or late is, of course, somewhat arbitrary since the chromosomes go through a continuous process of shortening and thickening. Figure 1*B*, *C* and *D* represent the earliest stages which would be called early, mid and late prophase, respectively). By 35-40 hours at room temperature almost all nuclei had started to divide and many of the mitoses were complete.

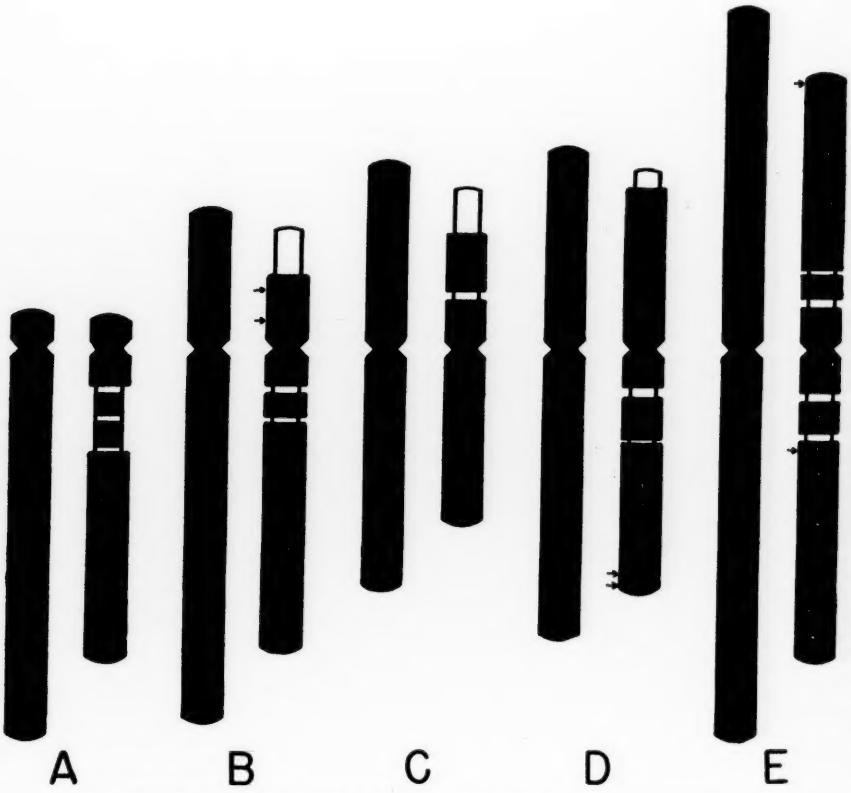
The number and distribution of differential segments. In all metaphases and early anaphases studied in 6°C. cultures, regardless of the length of either the initial culturing, at room temperature or the cold treatment, some chromosomes showed differentiated segments. For the 40 metaphases or early anaphases studied for all 6°C. treatment the average number of differentiated segments was 8.1 per haploid set of chromosomes (see Table II). Although the numbers are small, there is some indication from the figures in Table II that the longer the cold treatment the greater the differentiation, at least within the times studied. When the numbers of differentiated segments are expressed as percentages of a standard number (17 per cell) for comparison with the results of earlier experiments on root-tip mitoses (Wilson and Boothroyd[20]) it is found that they lie between those for root-tip cells after 72 and 96 hours at 3°C., the average figure for the pollen tubes, 48 percent of standard, being close to the 50 percent of standard seen in root tips after 96 hours at 3°C. (the treatment which gave the greatest degree of differentiation).

The positions of the differentially reactive segments were found by Wilson and Boothroyd[20] to be almost constant in their *Trillium erectum* material collected in Quebec, although not all segments were visibly differentiated in any one cell. Similarly Bailey[1] has reported that plants of *T. erectum* from Tennessee also have a fairly uniform pattern of differential segments with a few minor variations, but has drawn attention to a number of differences between the positions of the differentially reactive regions in the chromosomes of the Quebec and Tennessee populations. As a result of these differences he has concluded that "the pattern of differential segments produced by cold treatment cannot be employed as a valid or even useful taxonomic indicator for distinguishing species." The present author disagrees with this conclusion for several reasons. First, the differences in the patterns of differential segments between the Quebec and Tennessee populations of *T. erectum*, as noted by Bailey, may in several instances be more apparent than real (see below). Second, consistent differences in the patterns of the differential segments in the chromosomes of different species are possible even when there is a considerable amount of intraspecific variability. In such cases the patterns may not only help us to distinguish between species, but also, through a study of the differences in

TABLE I. The Relative Frequencies of Various Mitotic Stages After Different Times in Culture

Hours of culturing at room temperature	at 6°C	Interphase		Stages of Mitosis: Prophase* Early		Prophase* Mid		Prophase* Late		Metaphase		Anaphase		Telophase		Total Cells
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
20-21	0	168	25.3	426	64.2	63	9.5	7	1.1	0	0	0	0	0	0	664
"	212 (3°C)	47	22.1	140	65.7	23	10.8	3	1.4	0	0	0	0	0	0	213
"	92-173	157	15.5	361	35.6	271	26.7	155	15.3	30	2.9	26	2.6	15	1.5	1015
24	0	74	13.3	373	67.0	86	15.5	21	3.8	1	0.2	1	0.2	0	0	556
"	120-185	76	14.0	132	24.2	124	22.8	110	20.2	39	7.2	16	2.9	47	8.7	544
29-30	0	33	14.1	123	52.5	27	11.5	36	15.4	4	1.7	5	2.1	6	2.6	234
"	142-167	33	18.6	21	11.8	51	28.7	42	23.6	3	1.7	10	5.6	18	10.0	178
34-35	0	3	1.7	60	33.8	39	21.9	28	15.7	21	11.8	15	8.4	12	6.7	178
"	158	17	9.7	24	13.7	18	10.3	21	12.0	25	14.3	18	10.3	52	29.8	175
40-43	0	0	0	57	20.6	72	26.1	66	23.9	26	9.4	19	6.9	36	13.1	276
50	72(3°C)	4	2.7	16	10.9	37	25.2	40	27.2	9	6.1	4	2.7	37	25.2	147

*Figure 1 *B*, *C* and *D* represent the beginnings of early, mid and late prophase, respectively.



DISTRIBUTION OF HETEROCHROMATIN IN *TRILLIUM ERECTUM*

Figure 2

Pollen tube chromosomes of *T. erectum* showing for each of the five chromosomes, the appearance at room temperature on the left and at 6°C. on the right. Relative lengths are indicated, but not diameters. The average sizes and positions of the heterochromatic segments are shown. Arrows indicate the approximate positions of additional segments of heterochromatin found by Wilson and Boothroyd[20] in their more extensive root-tip material.

pattern between populations within a species, to gain some insight into the course of intraspecific diversification and the evolution of varieties and subspecies. Third, differential patterns have already been used to advantage in identifying the chromosome sets and determining the relationships of several Japanese species of *Trillium* (Haga and Kurabayashi[10]).

In the present investigation no differential segments were found in the pollen-tube chromosomes which had not previously been found in root-tip chromosomes in the earlier study (Wilson and Boothroyd[20]). Figure 2 shows the distribution of differential segments as found in pollen-tube mitoses of *T. erectum*. The 15 segments illustrated here correspond to those most frequently found in the root-tip chromosomes. They form what may be considered to be the basic pattern for the Quebec population of the species, although not all these segments will be differentiated in every metaphase of cold-treated material. That the remaining six differential regions illustrated in Wilson and Boothroyd's[20] Figure 11 were not found in the present material is not surprising, in view of the much smaller number of plants used in the present study and the rarity with which these segments were differentiated in the extensive root-tip material. The positions of these six segments are indicated by arrows in Figure 2.

When the pattern as shown in Figure 2 is compared with that for the Tennessee population as illustrated in Bailey's[1] Figure 5 a marked similarity is apparent. As seen in Figure 2 and Figure 1 *I*, chromosome A has three differential segments in the proximal half of the long arm, although frequently only two and occasionally only one of them may be visibly differentiated in any particular cell (occasionally the "euchromatin" between the heterochromatic bands becomes differentiated, although to a lesser degree than the heterochromatin, to give one long differentiated segment; see Figure 1 *F*). Chromosome B most commonly shows two regions differentiated; a long terminal one in the short arm and a shorter interstitial one in the proximal part of the long arm (Figure 1 *G*). Occasionally a second short interstitial segment is seen in the long arm just distal to the first. Chromosome C almost always has one differentiated segment, a terminal one in the short arm (Figure 1 *J*), and occasionally a very short interstitial one in the same arm. Chromosome D commonly has a terminal portion of the short arm differentiated as well as a short interstitial region in the proximal part of the long arm (Figure 1 *H*). A second differentiated segment was seen just distal to the latter in one case. Chromosome E most frequently has two visible differential segments, one each side of the kinetochore. However, in many cells there are two such regions in one arm and one in the other, while in about one cell in ten both arms have two differentiated regions. The pattern of regions most often differentiated in the present material corresponds very closely with that illustrated by Bailey[1] in his Figure 5 at *A'*, *B*, *C*, *D* and *E*. Most of the variation in pattern may be explained by assuming a variable response of the differentially reactive segments to the influence of low temperature. On this basis the patterns illustrated here in Figure 2 and in Bailey's Figure 5 merely represent commonly occurring partial manifestations of the potential pattern more fully represented in Wilson and Boothroyd's[20] Figure 11. Thus the differences between populations are slight, and the basic pattern of differential segments appears to be a useful taxonomic character.

Bailey[1] noted that the kinetochore regions were clearly differentiated from the adjacent euchromatin at low temperatures. The present author, while also noting this condition of the kinetochores, has not included them in the pattern of differential segments for the following reasons: (a) the special character of the kinetochore, (b) the fact that the kinetochore is visibly differentiated at higher temperatures, and (c) any agent which causes supercontraction of the chromosomes tends to increase the differentiation between the kinetochore and the rest of the chromatin, although most such agents do not show up the "differential segments" of heterochromatin.

Chromosome contraction. Contradictory reports are to be found in the literature on the relationship, if any, between chromosome contraction and the cold-induced differentiation of heterochromatin in *Trillium*. Wilson and Boothroyd[20] showed clearly that in their *T. erectum* material there was a reduction in the over-all length of the chromosome complement with cold treatment (3°C.), and that the euchromatin was supercontracted in relation to the heterochromatin. The difference in the degree of contraction of eu- and heterochromatin was proportional to the number of heterochromatic regions visibly differentiated. Bailey,[1] using both the aceto-carmine and Feulgen techniques, confirmed the fact that cold produced a differential contraction. Bowen and Wilson[3] also reported a relationship between contraction and differentation. Resende, Lemos-Pereira and Cabral[15] did not find any such relationship ("On remarque l' indépendance entre la différentiation hypochromatique et la contraction chromosomique"). However, they reported that the degree of differentiation became greater with lower temperatures and at the same time showed chromosome contraction to be greater at these lower temperatures. Darlington and La Cour[5, 6] and La Cour[14] have stated very definitely that no difference in contraction is involved.

Measurements of the total length of the chromosome complement in pollen-tube metaphases of control and cold-treated material showed that the chromosomes were significantly shorter at 6°C. than at room temperature. The average length of the complement was found to be 145.3 μ in the control material and 120.3 μ in the cold treated. These two values differ significantly ($t=2.828$, D.f.$=47$, $P=<0.01$).

More important than the total length of the complement for an understanding of the differentiation of the heterochromatic segments are the length relationships of hetero-and euchromatin. Because the heterochromatin is not visibly differentiated at room temperature this relationship cannot be measured directly. However, in *T. erectum* the short arms of chromosomes B and C have long segments of

TABLE II. The Number of Differential Segments Observed in Pollen Tube Metaphase Chromosomes After Various Cold Treatments

Hours at 6°C.	Number of Nuclei	Total Differential Segments	Mean Differential Segments per cell	Percentage of Standard Number*
92-120	5	36	7.2	42
142-147	16	121	7.6	45
158-173	19	165	8.7	51
Total (92-173)	40	322	8.1	48

*The standard number of differential segments used here is 17 per cell, for comparison with the results of Wilson and Boothroyd[20] who used a standard number of 34 per root-tip cell.

heterochromatin and the long arm of chromosome C is wholly euchromatic. If there is any marked difference in the degree of contraction of eu- and heterochromatin under the influence of cold it might be expected that the ratios of the lengths of the B and C chromosome short arms to that of the C long arm would be different in cold-treated and control material. When these ratios were calculated significant differences were found. The ratio of the short to the long arm of chromosome C was 0.800 for control and 0.915 for cold-treated material ($t=3.221$, $P=<0.01$). The ratio of B short arm to C long arm was 0.591 for control and 0.693 for cold-treated ($t=2.267$, $P=0.02$-0.05). These findings confirm those of Wilson and Boothroyd[20] that arms with a long differentiated segment of heterochromatin were relatively longer than euchromatic arms. In the light of this additional evidence of the occurrence of a differential contraction of eu-and heterochromatin the author is inclined to discount the statements of Darlington and La Cour that no such difference occurs, at least until they publish measurements. Indeed, their only published evidence to date, their camera lucida drawings, indicates that in their material also a differential contraction occurred (e.g. Darlington and La Cour[6]: *T. sessile*, chromosome B short arm/long arm=0.453 in control and 0.553 in cold-treated material of the same plant. These ratios are, however, based on drawings of a single pair of chromosomes in each case and may not be representative). Smith[17], working with the beetle *Tribolium*, has concluded from measurements of the X and Y chromosomes that "differential reactivity in *Tribolium* is primarily a matter of relative density of spiralization."

Thus it may be stated that differentiation of the heterochromatin in *Trillium* by low temperatures (6°C. in the present material) is associated with a differential contraction of the euchromatic and heterochromatic parts of the chromosomes, the heterochromatin being undercontracted relative to the euchromatin. This difference in contraction does not, however, seem to be sufficient to account for all differences in chromaticity, which probably indicate differences in nucleic acid content, as proposed by Darlington and La Cour[5, 6, 7].

The stage of mitosis at which differentiation may be induced. From the present study it is apparent that differentiation of the metaphase chromosomes appears when cold treatment is begun during prophase. Cultures kept at 6°C. for 92-142 hours, following 20-21 hours at room temperature, contained a few cells in metaphase and two in very eary anaphase, but no later stages. All the metaphases and both anaphases showed clear differentiation of the heterochromatic segments of the chromosomes and averaged eight such segments per cell, or per haploid complement (Figure 1 *G*, *H* and *J*). Since the metaphases and anaphases studied were the most advanced stages of division on the slides and since most of the cells were in prophase at the start of the cold treatment (a few even in mid or late prophase, see Table I), it can safely be concluded that the metaphases seen after cold treatment were in prophase when the treatment was begun, and most probably at least in mid prophase.

Further evidence to support this conclusion that differentiation of metaphase chromosomes can be induced by cold treatment begun at mid prophase may be obtained from material cultured 34-35 hours at room temperature before refrigeration. After 158 hours at 6°C the metaphases averaged 9.2 differential segments per complement. Since only a small proportion of the cells would still have been in interphase at the start of the cold treatment (see Table I) it is highly probable that the metaphases studied were well into prophase at that time. Unfortunately all cultures refrigerated after 40-43 hours at room temperature, at which time no interphases remained, had bacterial or fungal infections which rendered them useless.

Finally, all metaphases studied in cultures kept at 6°C. showed some differential segments regardless of the length of the cold treatment or of the previous incubation at room temperature. Since the lengths of the cold treatments varied from 92 to 173 hours it seems safe to assume that the cells which were in metaphase at the end of the treatment were in various stages of mitosis from interphase to late prophase when cold treatment started.

One series of cold treatments was given at 3°C. for 72 hours after 50 hours at room temperature. The long incubation at room temperature would ensure that few, if any generative nuclei would still be in interphase. Probably the four interphases found (Table I) were incapable of division. As has been pointed out above, 3°C. appears to arrest mitosis in the pollen tubes of *Trillium erectum*. Therefore the cells studied after 72 hours at 3°C. were probably in the same stages of mitosis as they had been at the start of the cold treatment. The twelve metaphases which could be analyzed showed a definite differentiation of the heterochromatin (see Figure 1 *F*), although usually it was not as sharp or clear-cut as with 6°C. treatments. Length measurements were possible in only three of these cells, but these gave an average length for the chromosome complement of 121.2 μ. The number of measurements is too low for a statistical analysis, but the average is so close to that for the 6°C. material (120.3 μ) and so much below that for the controls (145.3 μ) that it appears to be clear evidence of supercontraction of the metaphase chromosomes at 3°C. This is supported by observations on other cells which were not clear or flat enough to measure, but in which the chromosomes appeared shorter and thicker than in control ma-

terial. Thus we may say that a slight degree of differentiation of the heterochromatin may be induced in chromosomes which are arrested at metaphase and become supercontracted.

Theories concerning the differential reaction of chromosomes to cold. Three main theories have been advanced concerning the differential reaction of various parts of the chromosomes to low temperatures, namely (a) the nucleic acid starvation theory of Darlington and La Cour, (b) the nucleolar olistherozone theory of Resende *et al.*, and (c) the differential contraction theory of Wilson and Boothroyd.

(a) Nucleic acid starvation. Darlington and La Cour[6, 7] believe that when interkinesis occurs at a low temperature an insufficient amount of nucleic acid is synthesized by the cells. During the following mitosis the chromosomes are starved of nucleic acid and the genetically inert heterochromatin, which has less affinity for it than has euchromatin, remains undercharged with nucleic acid, although showing a normal degree of metaphase contraction. In support of this theory they state[7] that ". . . . in *Trillium*, nucleic acid starvation requires three days to affect the metaphase of mitosis in roots. Even at a low temperature this period must compmise most of the resting stage. In pollen grains chilling has to be much longer. . . . . The starvation effect therefore acts long before the prophase when the chromosomes are actually taking up their nucleic acid." This claim is in part refuted by Resende, Lemos-Pereira and Cabral[15], who found a standard treatment of 15 hours in the cold was effective in inducing differentiation in the chromosomes of *Trillium sessile* and who quote Geitler[8] to the effect that six hours is sufficient for *Adoxa*. Wilson and Boothroyd[19, 20] found treatments as short as half an hour caused visible differentiation in the chromosomes of *Trillium erectum* root tips, although maximum differentiation required four days at 2°C. They believed that the very short cold treatments which were effective indicated that chromosomes which were already in prophase when cold treatment was started might show differentiated segments at metaphase. Since the rate and duration of mitosis at this temperature was unknown, this coud not be taken as established fact.

The nucleic acid starvation theory of the cause of the differential reactivity of chromosomes to cold is still maintained by La Cour[14] in spite of the difficulty of reconciling it with the above evidence on the ability of very short cold treatments to cause differentiation. That it must be modified, if not discarded, is apparent in the light of the present evidence that differentiation of the heterochromatin in metaphase chromosomes may be induced by cold treatments begun as late as mid prophase (and probably even at late prophase or metaphase itself). Recent work of Howard and Pelc[12, 13] indicates that nucleic acid synthesis occurs during interkinesis and is completed before the start of mitotic prophase in *Vicia faba* roots. If this is also true of *Trillium*, then cells which had completed interphase at normal temperatures should have the full amount of nucleic acid. Consequently some explanation other than a reduction in the total amount of nucleic acid must be sought for the induction of differentiation by cold treatments begun at prophase.

(b) Nucleolar olistherozones. Resende, Lemos-Pereira and Cabral[15] think it improbable that the differentially reactive segments represent heterochromatin. They believe that "these ten olistherozones of *Trillium sessile* var. *rubrum* must be in their totality or in part nucleolar zones," and suggest that, since *Trillium* and most other plants in which this peculiar effect of temperature on the chromosomes has been demonstrated are native to cold climates, the hypochromaticity of these special regions should not be considered as resulting from the action of cold. It should rather be considered as the normal condition of these segments, similar to the nucleolus organizers in other plants, and that high temperatures cause abnormal disappearance of the differentiation. As pointed out by La Cour,[14] there appears to be little evidence to support the view that the differential segments are nucleolar zones. Contrary evidence is seen in the fact that there are far fewer nucleoli formed than differentially reactive segments, yet these latter all appear to behave in essentially the same manner. Also, the ends of certain chromosomes which have been seen to organize nucleoli do not become differentiated by cold.

(c) Differential contraction. Wilson and Boothroyd[20] showed that in *Trillium erectum* root tips the euchromatin was supercontracted and the heterochromatin undercontracted by cold-treament, as compared with the degree of contraction at normal temperatures. The direct correlation between the difference in the degree of contraction and the number of heterochromatic segments visibly differentiated after various times in the cold led to the conclusion that "it . . . . seems probable that the observability of differential regions depends largely on the degree to which their contraction differs from that of the rest of the chromosomes."

The results of the present study show that in pollen-tube chromosomes, as in those from root tips, arms with long heterochromatic segments are less contracted after cold treatment than are wholly euchromatic arms. It seems likely, therefore, that at the lower temperature the normal prophase contraction of heterochromatin is decreased, while that of the euchromatin is enhanced. The slight amount of differentiation seen in pollen-tube metaphases at 3°C. could result from the supercontraction of the euchromatin. The fact that Resende, Lemos-Pereira and Cabral[15] found differentiation of the heterochromatin (their

"olistherozones") without supercontraction of the chromosome complement might result from the under-contraction of the heterochromatin being sufficient to cancel the effects of the supercontraction of the euchromatin, so that the total chromosome length remained unchanged. This may be seen in Wilson and Boothroyd's[20] 12 hour cold treated material in which the total length of the chromosome complement was the same as in the controls, but some differentiation was seen within the chromosomes (loc. cit. Table III and Fig. 15).

If cold induces a visible differentiation of various parts of the chromosomes by preventing heterochromatin from contracting normally, while allowing the normal contraction, or even supercontraction, of euchromatin, then the stage of mitosis at which cold treatment was started would be expected to influence the degree of differentiation. The earlier in prophase that cold treatments were begun the greater would be the difference in contraction which could occur. If treatment were begun in metaphase or prometaphase, when normal contraction of the chromosomes is almost completed, then differentiation would be possible only by supercontraction of the euchromatin. The present findings on pollen-tube mitoses, as well as those of Wilson and Boothroyd[19, 20] on root tips, appear to fit this hypothesis of differentiation by differential contraction.

Summary

Pollen-tube mitosis in *Trillium erectum* was stopped at a temperature of 3°C., but proceeded slowly at 6°C.

At 6°C. heterochromatic segments of the chromosomes appeared narrower and less deeply stained than the euchromatin, whereas at normal room temperatures no such difference could be seen. The distribution of these segments was found to be similar to that previously reported for root-tip chromosomes.

The total length of the chromosome complement at metaphase was considerably less at 6°C. than at room temperatures.

Chromosome arms with long segments of heterochromatin were less contracted by cold than were completely euchromatic arms, indicating that heterochromatin is undercontracted relative to euchromatin.

Differentiation of the heterochromatin in metaphase chromosomes may be induced by cold treatment started during prophase.

It is concluded that visible differentiation of the heterochromatin from the euchromatin is determined largely by the difference in the degree to which they are contracted. The earlier in prophase that cold treatment is begun, therefore, the more extreme should be the degree of differentiation induced.

Literature Cited

1. Bailey, P. C. Differential chromosome segments in *Trillium erectum* L. *Bull. Torrey Bot. Club* 76: 319-336. 1949.

2. Boothroyd, E. R. Differential contraction of the chromosomes of *Trillium* in relation to the division cycle. *Rec. Gen. Soc. Amer.* 18: 77. 1949.

3. Bowen, C. C. and G. B. Wilson. Some factors correlated with differential reactivity in the somatic chromosomes of *Trillium*. *Rec. Gen. Soc. Amer.* 19: 90. 1950.

4. Callan, H. G. Heterochromatin in *Triton*. *Proc. Roy. Soc.* (Lond.) B. 130: 324-335. 1942.

5. Darlington, C. D. and L. La Cour. Differential reactivity of the chromosomes. *Ann. Bot.* n.s. 2: 615-625. 1938.

6. ————. Nucleic acid starvation of chromosomes in *Trillium*. *Jour. Gent.* 40: 185-213. 1940.

7. ————. The detection of inert genes. *Jour. Hered.* 32: 115-121. 1941.

8. Geitler, L. Temperaturbedingte Ausbildung von Spezialsegmenten an Chromosomenenden. *Chromosoma* 1: 554-561. 1940.

9. Haga, T. Morphological reaction of chromosomes to low temperature. *Jap. Jour. Genet.* 20: 87. 1944.*

10. ————and M. Kurabayashi. Genome analysis in the genus *Trillium* on the basis of differential reaction of the chromosomes. *Jap. Jour. Genet.* 22: 8. 1947.*

11. ————and————. Chromosomal variation in *Trillium kamtschaticum*. *Jap. Jour. Genet.* 23: 12-13. 1948.*

12. Howard, Alma, and S. R. Pelc. Nuclear incorporation of P^{32} as demonstrated by autoradiographs. *Exptl. Cell Res.* 2: 178-187. 1951.

13. ————. Synthesis of nucleoprotein in bean root cells. *Nature* 167: 599-600. 1951.

14. La Cour, L. F. Heterochromatin and the organization of nucleoli in plants. *Heredity* 5: 37-50. 1951.

15. Resende, F., A. de Lemos-Pereira and A. Cabral. Sur la structure des chromosomes dans les mitoses des meristemes radiculaires. *Portug. Acta Biol.* 1: 9-46. 1944.

16. ————, Juana Salord and L. Leite-Rio. Structure of chromosomes as observed in root tips. *Portug. Acta Biol. Ser.* A 1: 412-415. 1946.

17. Smith, S. G. The evolution of heterochromatin in the genus *Tribolium* (*Tenebrionidae*: *Coleoptera*). *Chromosoma* 4: 585-610. 1952.

18. Wilson, G. B. The Venetian turpentine mounting medium. *Stain Tech.* 20: 133-135. 1945.

19. ————and E. R. Boothroyd. Studies in differential reactivity. I. The rate and degree of differentiation in the somatic chromosomes of *Trillium erectum* L. *Can. Jour. Res. C.* 19: 400-412. 1941.

20. ———— and ————. Temperature-induced differential contraction in the somatic chromosomes of *Trillium erectum* L. *Can. Jour. Res. C.* 22: 105-119. 1944.

*English Summary in *Heredity*, Dec. 1950.

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THE EFFECT OF LIGHT ON THE EXPRESSION OF HETEROSIS

In *Antirrhinum majus* L.

W. J. Haney, J. B. Gartner and G. B. Wilson*

It is generally conceded that hybrids between various highly inbred lines of the snapdragon, *Antirrhinum majus* L. exhibit a marked degree of heterosis. Haney (unpublished) has investigated a large number of such hybrids and has found that, while many of them exhibit obvious heterosis, the degree of expression is by no means constant, even for the same hybrid, when raised at different times. Since such variation renders genetical study very difficult, the present investigation was undertaken partly with a view to determining whether or not some controllable extrinsic factor or factors were operating. Similar variation has been noted in other crops, notably tomato[1, 3] and corn[4] and has led to controversy concerning the general value of specific hybrids.

Materials and Methods

The inbred lines used have the following origins:

1. P_1 came from a single plant selection out of a strain of Rockwood's Pink Supreme secured from the George J. Ball Company, West Chicago, Illinois, and was subsequently inbred over five generations.
2. P_2 was similarly isolated and inbred from the variety Helen Tobin introduced by Dr. Harold White of the University of Massachusetts. Both inbred lines appear to be highly homozygous.

Three experimental greenhouse plantings were made using the two inbreds, their hybrid and a random selection of the F_2. In each case 350 seedlings of P_1, and P_2 and the F_1 and 1250 seedlings of the F_2 were benched. Planting distances were 3×3 inches. Height measurements were made in each case when the hybrids were approximately 10 centimeters high. Average dry weight of the P_1, P_2 and F_1 and F_2 plants were determined a few days later. Observations were made at this stage since experience has shown that at this planting distance crowding disturbs the relationships at later stages. Planting and recording dates are as follows:

Experiment 1: Seed was sown on December 1, 1951, and seedlings benched on January 15, 1952. Height measurements were made on February 5, 1952.

Experiment 2: Seed was sown on February 3, 1952; seedlings benched on March 28 and heights measured on April 26.

Experiment 3: Seed was sown on March 28, 1952; seedlings benched on April 9 and heights recorded on May 8.

So far as the readily controllable factors of nutrition and culture were concerned the three experiments were maintained under the same conditions. There was some variation in temperature and considerable variation in light intensity and total amount of solar radiation.

Observations

In all three experiments some degree of heterosis is exhibited by the F_1 as shown by

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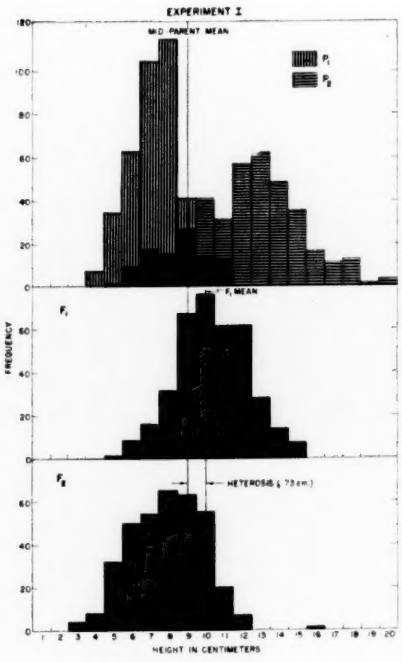
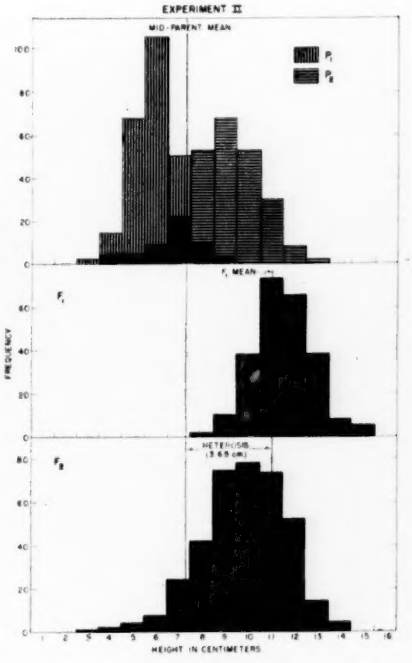


EXPERIMENT I

Figure 3

Frequency distribution of heights in centimeters of parents, F_1 and F_2 for Experiment I. Distance between mid-parent mean and F_1 mean is taken to represent degree of heterosis.



EXPERIMENT II

Figure 4

Frequency distribution of heights in centimeters of parents, F_1 and F_2 for Experiment II.

the displacement of the mean height and dry weights to the right of the mid-parent mean (see Figures 3, 4 and 5 and Tables I and II). Regardless of which criterion is used the amount of heterosis varies from experiment to experiment. Since there appeared to be some relation between the variation and light conditions an attempt was made to determine the degree of correlation between the expression of heterosis and solar radiation. Since no suitable records were available for the greenhouse itself, pertinent data were obtained from the U. S. Department of Agriculture, Hydrologic Station on the campus of Michigan State College. Averages for the periods during which the experiments were conducted were calculated and plotted against the degree of heterosis as determined both on the basis of height and dry weight differences (Figure 6). The resultant curves are (a) remarkably close

TABLE I. Average height in centimeters of 350 plants each of inbred (P_1 and P_2) and the hybrid (F_1) and segregating population (F_2) of *A. majus*.

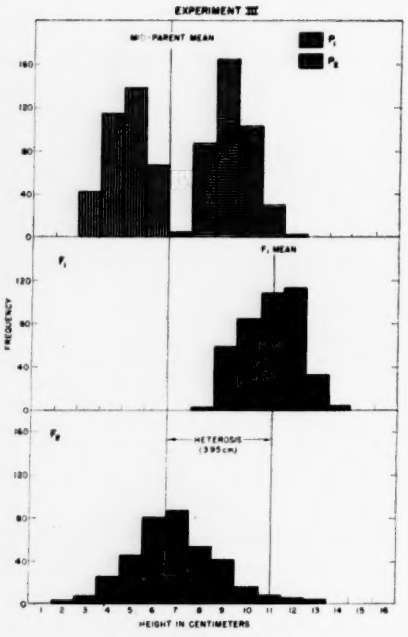
	Parent 1 (P_1)	Parent 2 (P_2)	Mid-parent mean	Hybrid (F_1)	(F_2)	Heterosis*
Exp. I	7.3	12	9.65	10.38	7.91	0.73
Exp. II	5.9	8.93	7.415	11.1	9.9	3.68
Exp. III	4.7	9.2	6.95	10.9	6.9	3.95

*Heterosis is the difference between mid-parent mean and F_1.

TABLE II. Average dry weight in grams of individual plants of Parent 1 (P_1) Parent 2 (P_2) and hybrid (F_1). Individual averages are based on the average of 350 plants.

	Parent 1 (P_1)	Parent 2 (P_2)	Mid-parent mean	Hybrid (F_1)	Heterosis*
Exp. I	0.216	0.357	0.286	0.401	0.115
Exp. II	0.231	0.206	0.216	0.570	0.354
Exp. III	0.12	0.16	0.14	0.6	0.46

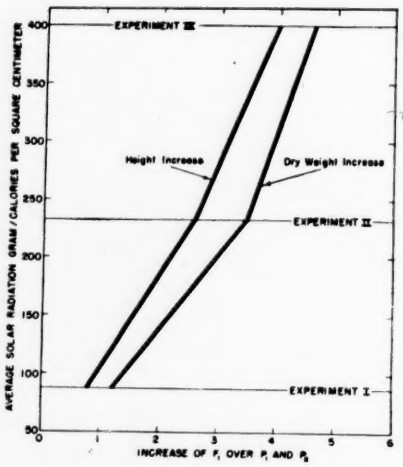
*Heterosis is the difference of the F_1 from the mid-parent mean.



EXPERIMENT III

Figure 5

Frequency distribution of heights in centimeters of parents, F_1 and F_2 for Experiment III.



RESULTS OF THE EXPERIMENT

Figure 6

Correlation between solar radiation and heterosis as measured both by height increase and dry weight increase of F_1 over parents.

to straight lines and (b) very nearly parallel thus showing a high degree of correlation between the expression of heterosis on both criteria used and solar radiation.

Discussion

There appears to be little doubt that the expression of heterosis in the snapdragon is dependent upon the amount of solar radiation to a rather marked degree. From the genetical point of view this means that any study designed to determine the number and kind of genes involved in heterosis must take due cognizance of the necessity for rigid environmental control. From the practical point of view it would seem that "blanket" claims for the superiority of hybrid snapdragons should not be made without reference to the precise conditions of culture required. The operation of the photochemical mechanism involved in the relation between solar radiation and heterosis is outside the scope of this paper. However, evidence[2] to be published later, indicates involvement of indoleacetic acid production and utilization.

It would be naive in the extreme to suppose that the amount of solar radiation is the paramount factor in the expression of heterosis generally or even in all cases in the snapdragon. One would scarcely expect light to be a major factor at all in animals. However, that light may be a fairly important factor in plants generally is suggested by Whaley's work[4] with inbred and hybrid corn in which he was able to show that light intensity was correlated with the expression of heterosis.

Literature Cited

1. Currence, T. M., R. E. Larson and A. A. Virta. A comparison of six tomato varieties as parents of F_1 lines resulting from the fifteen possible crosses. *Proc. Amer. Soc. Hort. Sci.* 45:349-352. 1944.

2. Gartner, J. B. Interrelation of response to indole acetic acid, duration and intensity of light and heterosis in the snapdragon (*Antirrhinum majus* L.) Ph.D. thesis, Michigan State College. 1952.

3. Larson, R. E. and T. M. Currence. The extent of hybrid vigor in F_1 and F_2 generations of tomato crosses. *Minn. Agr. Exp. Sta. Tech. Bull.* 164. 1944.

4. Whaley, W. G. Heterosis. *Bot. Rev.* 10:461-498. 1944

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A NEW EYE ABNORMALITY IN THE HOUSE MOUSE

L. Butler and D. A. Robertson*

IN 1949 a number of eye abnormalities were noticed in one strain of mice in our laboratory. This strain of *D b A wa-2* had been obtained from McGill University, in which colony the eye abnormality "lens rupture" had been found by Fraser and Herer.[4] Some of the abnormalities were similar to lens rupture, while others, particularly squint (Figure 7 *A*) were not. Furthermore, lens rupture is a recessive with complete penetrance, whereas these abnormalities showed incomplete penetrance. It appeared, therefore, that either we were dealing with varied expressions of the lens rupture gene, or else a new mutant was present in this stock. In order to distinguish between these two hypotheses, a breeding program was set up and all animals were classified for the four types of eye abnormalities described below.

Phenotypic Classes of Eye Abnormalities

Squint—has one or both eyes closed or partly closed so that the mouse appears to be peering or squinting in a bright sunlight. The eyelids are usually formed, but in extreme cases they stay at the rudimentary stage seen in the twelve-day old mouse embryo. The eyeball is present but varies from slightly reduced to greatly reduced in size. There is a large amount of suppurative matter between and about the eyelids, and around the reduced eyeball. The optic nerve and muscles of the eyeball are formed but are reduced in size. The variation in the formation and completion of the internal structure of the eye, is apparently related to the degree of adhesion between the eyelids.

At birth, when the eyelids of the normal individual are completely covered by the as yet united eyelids, there is an obvious opening between the eyelids of the abnormal mouse. By the second day after birth, there is a definite suppuration between the eyelids, and under them, between the eyelids and the eyeball. Sometimes at this stage, or by a few days later, there is a welt of scab tissue formed between the eyelids, which persists for varying periods but is usually absent by weaning time. This is similar to the description by Loeffler[6] of "eyes open at birth."

Cataract eye is always associated with the squint complex, and is considered to be a less complete expression of that gene. The eyelids are of normal disposition and formation. The eyeball is reduced in size, though the reduction is not as great as with the squint expression. There is some suppuration under the eyelids and about the eyeball. The conjunctiva of the eyeball is more obviously connected with the bodies of pustulent tissue, and is opaque over the surface of the eyeball.

As an expression of the squint complex, the abnormal condition is detectable at birth though it is not possible to differentiate between the squint or cataract individual at birth.

Occasionally, a squint individual will change in expression to a cataract type. One mouse (M97) was classified at 30 days as a squint individual, but by 90 days the eyelids had digressed necessitating its reclassification as cataract.

Limpid Pool—was first detected in the unpigmented eye of the albino mouse. The first sign of the abnormality was a small opacity that appeared in the depths of the eyeball. As the abnormality developes, there is an apparent consolidation of the vague whitish area into a definitive, opaque white orb in a comparatively anterior region of the eyeball. Gross microscopic examination determined that this structure was the

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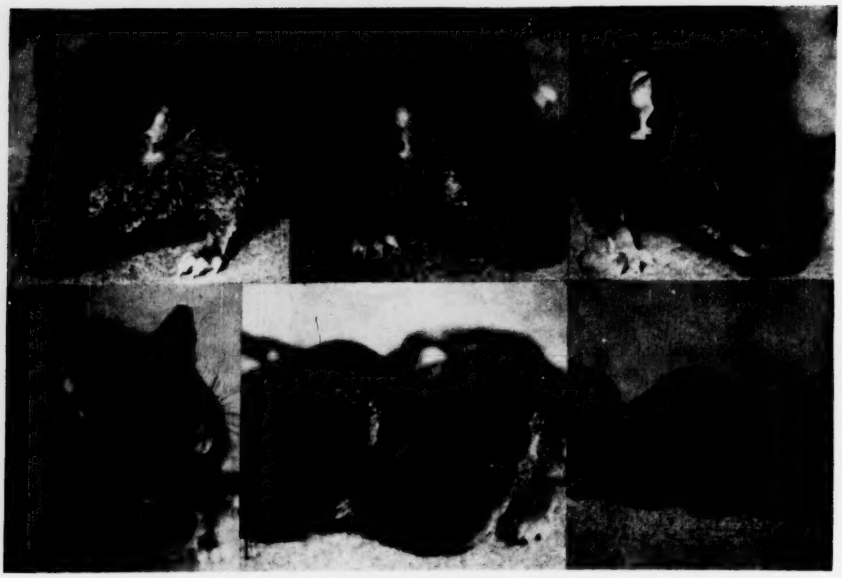

A NEW EYE ABNORMALITY IN THE HOUSE MOUSE

Figure 7

A—Mouse with squint cataract, showing eyes completely closed. *B*—Mouse showing squint cataract, with eyes partly open. *C*—Squint cataract mouse showing cataract. *D*—Normal litter mate. *E*—Two seven-day-old squint cataract mice. Left mouse has normal eyes, closed at this age, the mouse on the right is abnormal with eyes open. *F*—Day-old mice with open instead of closed eyes.

remnant of the lens after the "lens nucleus" had been extruded.

About 9 percent of the limpid pool mice also showed pin cataract. This condition was later determined to be the result of the extrusion of the "lens nucleus" into the anterior chamber of the eye, where it appeared as the small, white "pin head" described under pin cataract.

The incidence of pin cataract in the albino eye, coupled with the discovery of several "limpid pool" abnormalities in the pigmented eye of the colored mouse, led to an investigation of the "limpid pool" incidence in the colored eye.

The observation of the eye of the anaesthetized mouse, using atropine sulfate as a dilatant, and an ophthalmoscope with a fine beam of light, determined that all colored mice in the limpid pool strain which under ordinary observation had been classified as normal should have been classified as abnormal. The pigmented iris masks the opaque lens making classification more difficult.

Pin cataract was first observed in mice that were 30 days old, and 70 percent of the mice in this class could be identified by the time they were 60 days old. The other 30 percent show great variability in the age of onset, one mouse showing no signs of pin cataract until he was 175 days old. Pin cataract first appeared as a small round, pearl-grey or white opaque body in the anterior chamber of the pigmented eye of the colored mouse. As this "pin-head" was silhouetted against the dark iris, the abnormal eye was readily noted in the affected individual. Efforts to breed a true line proved futile since a great many normal individuals were obtained, while it was eventually noted that albino individuals appearing in this predominately colored strain were limpid pool.

Frequency and Laterality

In the first experiments abnormal were mated with abnormal, and it was found that from one to four types could occur in any litter. The classification of the young gave the following data:

Squint	45
Cataract	131
Limpid pool	360
Pin cataract	82
Pin cataract and limpid pool	38
Squint and cataract	52
Squint and limpid pool	1

Of the squint individuals only three were unilaterals although in bilaterals the expresson was more severe in the left eye than in the right. Eleven of the cataract mice were unilaterals, and of these, eight had cataract in the left eye only. Fifty-two mice had squint in one eye and cataract in the other. In 32 of these mice, the squint manifestation appeared in the left eye. This large class of mixed categories indicates close association between these two types.

The limpid pool mice were completely bilateral while in the case of pin cataract 19 were unilateral. The 38 which showed pin cataract and limpid pool were first classified from limpid pool and then as they became older were reclassified as pin cataract. This indicated that these two categories are associated.

Genetic Results

As a result of the above criteria, squint and cataract were grouped together in one complex and pin cataract and limpid pool were treated as another complex. Because of the similarity of pin cataract to the lens rupture, an outcross was made to *lr lr* mice. The resulting offspring all showed limpid pool and pin cataract, so this complex is the same as the previously described lens rupture. The character can be classified at an earlier age by making use of the limpid pool phenotypic expression.

When squint or cataract is outcrossed to *lr lr* the F_1 is normal, the F_2 segregates into a quarter lens rupture and a few (2 in 40) squint. This proves that this complex is not genetically identical with lens rupture.

In producing a true breeding line for the squint-cataract complex, crosses were made within and between the various types with the results shown in Table I. These figures demonstrate that the four phenotypic classes appear in all five crosses. The cross of squint $\times$ squint is no more likely to produce squint, than is the cross of cataract $\times$ cataract. Crosses 3 and 4 are especially illuminating on this point since in both, the one parent is a mouse with squint in one eye and cataract in the other. In cross 3 the second parent was squint, while in cross 4 the second parent was cataract. In these crosses the proportion of squint offspring was 8 percent when the second parent was squint and 27 percent when the second parent was cataract. The cataract proportions show the same anomalous behavior being 59 percent cataract when the second parent is squint, and 40 percent cataract when the second parent is cataract. Because of these two crosses it was decided that further analysis of these data would be unproductive, and that differences between the five crosses should be treated as chance ones. If this is done, squint and cataract are treated as phenotypic variables of the same gene or genes.

Turning next to the total of each type as shown in Table I, we find that cataract is the most common expression of this complex, accounting for almost 50 percent of the individuals. This may be partly due to the fact that fewer crosses were made of squint $\times$ squint. The reason for this is that squint individuals are less robust and make poorer parents. If, however, we accept the logical conclusion drawn from crosses 3 and 4, that squint parents do not produce more squint offspring than do cataract individuals, then these totals can be accepted as the proportion of each type to be expected. Squint in both eyes, and squint in one eye with cataract in the other, occur with about equal frequencies. There were 54 normal mice some of which came from each cross. The normals were not evenly distributed between the five crosses (Chi square 13.2 with P of .01), there being more in the first two crosses than would be expected by chance. Whether this represents a difference in penetrance could only be determined by more extensive data. The penetrance shown by the total figures is 82.2 percent. Three normal females from a squint $\times$ squint mating were mated with a brother that was squint, and these produced three normals and 12 with squint or cataract. This indicates that we are probably dealing with lack of penetrance in these normal individuals. The three normal individuals in this cross could again be due to lack of penetrance.

Outcross to C57 Black

Squint was crossed with C57 and produced 68 young, all of which were normals, indicating that this abnormality is a recessive. The F_1 individuals were backcrossed to squint and produced 36 normal to 12 squint. The Chi square for this is 12.0 if complete penetrance is assumed, and 4.3 if 80 percent penetrance is taken. Both of these values indicate a deficiency of abnormal mice. Seeking an explanation for this we find that the backcross

TABLE I. Phenotypic expression of the squint-cataract complex

Cross	Normal	Squint	Cataract	Squint Cataract	Total
Cataract × cataract	23	9	41	10	83
Squint × Squint	7	2	8	3	20
Squint/cataract × squint	12	6	44	12	74
Squint/cataract × cataract	4	13	19	13	48
Squint/cataract × Squint/cataract	7	15	30	14	66
Total	52	45	142	52	291

is also segregating for waved-2, and the digenic ratio is:—26 *Wa* normals: *O Wa* squint : 10 *wa* normal : 12 *wa* squint. This immediately points to an association of waved-2 and squint. From these rather meager data, it would appear that squint only manifests itself in the presence of waved-2. This would give an expected ratio of 24:0:12:12 which is quite close to the actual ratio given above. Until we have larger numbers and have demonstrated that half of the Wa_2 individuals are homozygous for squint, this hypothesis cannot be substantiated.

In the F_2 from this cross the segregation gave: 81 normal: 14 waved with normal eyes : to 7 waved with squint/cataract. This again gives too few abnormal eyes (chi square 17.9, P of .0001). If, however, we accept the hypothesis advanced above, the expected become 76.5 : 19.1 : 6.4, which gives a Chi square of 1.68 and a P of .4.

Discussion

Various eye abnormalities in the house mouse have been reported by Chase,[1, 2] Strong,[8] Fraser and Herer,[4] and Loeffler,[6] but none appear to be phenotypically identical with squint cataract. The closest approach is the gene 0 for eyes open at birth, which appears phenotypically identical in young mice but apparently is dissimilar in adult mice. These genes have not been tested for independence except in the case of lens rupture, but since none of these abnormalities shows an association with waved-2 mutant, it can be safely assumed that they are not identical. The description of the waved-2 mutant by Crewe,[3] Keeler,[5] Reed,[7] does not mention any eye abnormalities. It would appear that this is a new mutant which only produces its effect in the presence of the waved-2 gene. This mutant is being provisionally called squint cataract with the symbol *sq*. Before we have final proof of the mode of operation of the squint cataract gene, it will be necessary to cross normal mice from the C57 backcross to be sure that their geneotypes are either *W'a-2 wa-2 sq sq* or *W'a-2 wa-2 Sq sq*. It will also be interesting to see how squint cataract behaves in the presence of waved-1, and in *oo* mice.

Summary

Eye abnormalities appeared in one strain of mice. These abnormalities could be classified into four categories, two of which were found to represent the known mutant lens rupture. The other two categories were grouped together as a gene entity called squint cataract and given the symbol *sq*. The line in which squint cataract appeared were all waved-2, and it was found on outcrossing to C57, that in the backcross and F_2 all squint cataract mice were also waved-2. Close examination of the limited data indicates that squint cataract segregates independently of waved-2, but that it only shows in the phenotype when waved-2 is present. Analysis of the squint cataract line shows this gene to have a penetrance of 81 percent, and no apparent rule for the three types of expression: squint, cataract, and squint in one eye and cataract in the other.

Literature Cited

1. Chase, H. B. Studies on an anophthalmic strain of mice. iii. Results of crosses with other strains. *Genetics* 27:339-348. 1942.

2. ————. Studies on an anophthalmic strain of mice. iv. A second major gene for anophthalmia. *Genetics* 29:264-267. 1944.

3. Crewe, F. A. E. Waved—an autosomal coat form character in the mouse. *Jour. Genet.* 27:95-96. 1933.

4. Frazier, F. C. and M. L. Herer. Inheritance and expression of the lens rupture gene in the house mouse. *Jour. Hered.* 41:2-7. 1950.

5. Keeler, C. E. A second rexoid coat character in the house mouse. *Jour. Hered.* 26:189-191. 1935.

6. Loeffler, L. Ueber eine mutation bei der weissen hausmaus ihre genetik und deren bedeutung fur die menschliche. Erblehre. *Zeitschr. Ind. Abstamm. u Verb.* 61:409-446. 1932.

7. Reed, S. C. Determination of hair structure. 1. The production of waved hair from genetically non-waved cells. *Jour. Exp. Zool.* 79:347-354. 1938.

8. Strong, L. C. Inheritance of cataract and allied eye defects in the house mouse. *Proc. VI Int. Cong. Gen.* 2:195. 1932.

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CYTOGENETIC STUDY OF A MUTANT MAIZE F_2 PLANT

From Homozygous Diploid × Tester

LEE FORD*

DURING 1951-2 a study was undertaken of homozygous diploid lines in comparison to inbreds crossed into the tester line 1205/L289, in regard to details of meiosis, pollen analysis, detailed study of microsporogenesis, and notation of meiotic irregularities, with particular emphasis on homozygosis. The following brief report was a by-product of the main problem.

Procedures

To obtain a cytogenetic comparison of F_1 from homozygous diploids with F_1 from standard inbreds, 1700 seeds from crosses involving one or more monoploids, homozygous diploids, and standard inbreds from 186 different progenies were planted at the Ash Avenue Experimental Plot at Ames in the spring of 1951. Microsporocytes were collected and examined by the aceto carmine smear technique. Any observable phenotypic irregularities were noted.

Irregularities were also studied in advanced generations of the various monoploid-derived hybrids. 49-BKQ, a plant from the cross of homozygous diploid H65 × "N" (Randolph's 43687-1 brown liguleless stock), segregated for a dwarf-type of growth and also for high pollen sterility. H65, the one parent of 49-BKQ, had arisen in a marked progeny of the double cross of (OS420 × M14) × (WF9 × WF22) and the pollen parent, "N," was a *B Pl C* R^g *Pr lg*. The F_1 and F_2 of 49-BKQ were quite irregular and for this reason several plants of the advanced generation were grown and examined.

Experimental Results

Analysis of 49-BKQ and its advanced generation show a range of pollen sterility from completely normal to completely sterile. Out of 60 plants, 11 had normal pollen, three had no pollen, 20 had 50 percent or more pollen sterility, ten had 25 percent or less pollen sterility, one had small pollen, and 12 were not checked. The rest of the plants never reached maturity. As many as possible of these plants were examined cytologically with interesting results. The series of plants from 4-16-1 to 4-16-10 are immediate self progeny of the original plant, BKQ, and those from 5-8-1 to 5-12-10 are of the advanced generation from BKQ.

In an attempt to throw further light on the BKQ series of plants, several crosses of 49-BKQ onto L289 testers were checked cytologically for aberrations. The only observation was that there was a small percentage of laggards at metaphase I resulting in micronuclei at telophase, plus one or two chromosomes heterozygous for knobs. The knob configurations were such that a deletion could have been involved.

Plant 5-8-6 appeared to have meiotic divisions giving an effect similar to a plant severely treated with an insecticide, (Figure 8 *A* and *B*). Occasionally islands of tissue appeared with as many as three or four hundred metaphase-stage chromosomes all clumped together in a huge mass of protoplasm with no organization whatsoever, (Figure 8 *C* and *D*). Similar behavior in wheat-rye hybrids was ascribed by Katterman[2] to cytomixis, and by Yasui[4] to the loss of gene (*s*) governing the meiotic processes. Other similar abnormalities were reported by Love[3] and Hollingshead[1].

Plant 5-9-4 showed 50 percent pollen sterility and cytological examination indicated possible inversion and/or deletion on the long arm of chromosome 2. Plants 5-9-5 and 5-9-10

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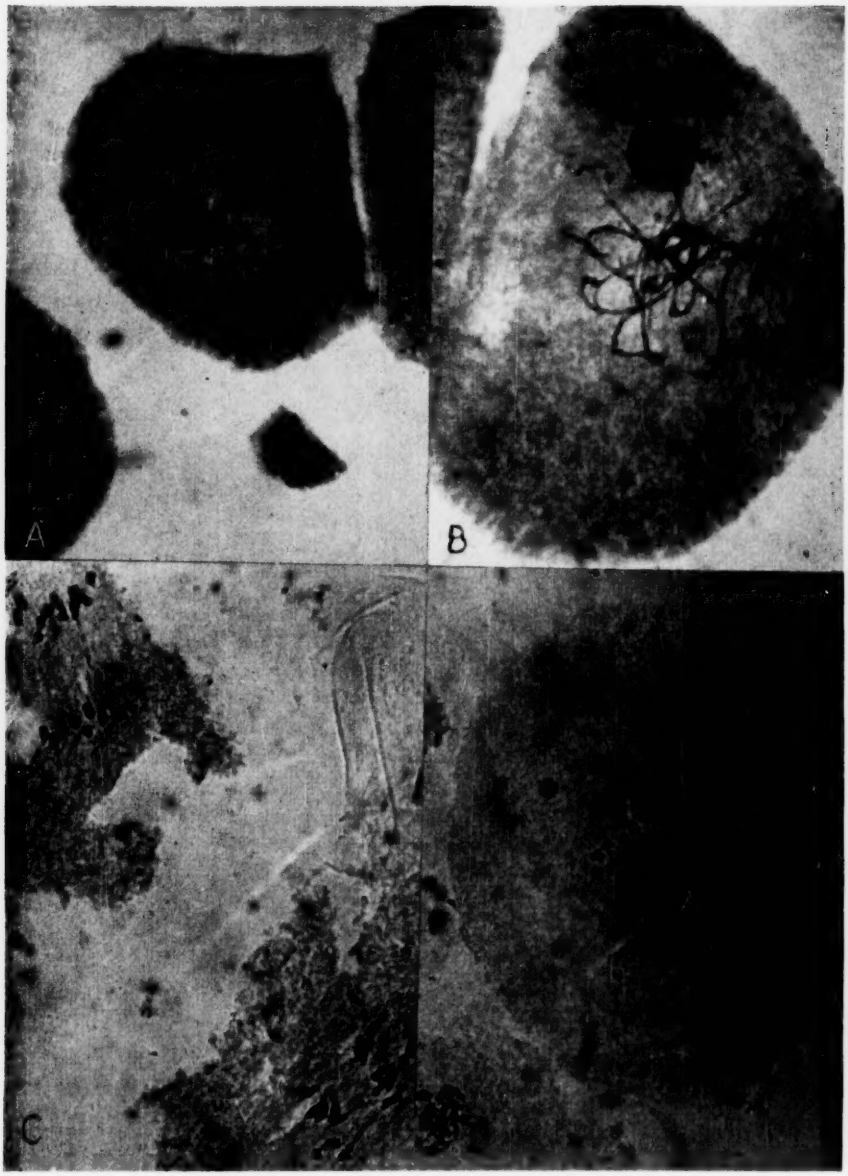
MEIOTIC ABERRATIONS

Figure 8

A and *B*—Plant 5-8-6 during meiotic division exhibiting an effect similar to a plant which has undergone severe insecticide treatment. *C* and *D*—shows islands of disorganized metaphase-stage chromosomes clumped together.



IRREGULARITIES DURING VARIOUS STAGES OF MEIOSIS

Figure 9

A—Plant 5-6-3 showing possible inversion of the long arm of chromosome 1. *B*—Two pachytene figures of Plant 5-10-6 showing the long arm of chromosome 1 or 2 heterozygous for a knob. *C*—shows the presence of laggards and bridges, but no other irregularities. *D*—Plant 4-16-2 showing laggards and bridges at anaphase II.

showed presence of laggards and bridges but no other irregularities were detectable (Figure 9 *C*). Plant 5-9-10 had 25 percent pollen sterility and there was no determination on plant 5-9-5. Plant 5-9-6 was examined in detail, showed 50 percent pollen sterility, bridges, laggards, non-pairing at diakinesis, but no recognizable inversions.

Plant 5-9-6 was very interesting. In one anther all stages of meiosis were present, including early prophase, diakinesis, anaphase I, metaphase I, telophase I, and anaphase II. It is very unusual to note this range of division occurring in a single anther or even in a single floret. Lagging and bridges plus fragments were observed at anaphase I. At diakinesis, nine pairs were observed together with two non-paired homologues. Lagging in metaphase plate formation was noted and also micronuclei at telophase I. Irregular anaphase II division resulted in 16 chromosomes going to two poles and four to the other two poles.

Plant 5-10-6 showed 50 percent pollen sterility. Diakinesis was regular but metaphase I showed laggards, anaphase I also showed laggards with one possible bridge figure. Metaphase II and telophase II also gave evidence of laggards. Two pachytene figures showed the long arm of chromosome 1 or 2 heterozygous for a knob plus a possible deletion (Figure 9 *B*).

Plant 5-11-2 showed 10 percent sterility, had laggards at metaphase and telophase I, plus two cells with two bridges and fragments at telophase I. Plant 5-6-3 showed a figure indicating possible inversion of the long arm of chromosomes 1 (Figure 9 *A*). Plant 5-6-4 showed many figures with ring of four and eight pairs at diakinesis. However, pachytene studies failed to show translocation configurations. Plant 4-16-2 showed laggards plus bridges at anaphase II (Figure 9 *D*). One figure fave an indication of possible deletion in the long arm of chromosome 1 or 2.

Thus a series of aberrations were found in the progeny of BKQ which probably included one or more inversions and deletions. Further study of the 49-BKQ progeny would undoubtedly throw further light on the details involved.

Literature Cited

1. Hollingshead, L. The occurrence of unpaired chromosomes in hybrids between varieties of *Triticum vulgare*. *Cytologia* 3:119-141. 1932.

2. Katterman, G. Ein Beitrag zur Frage des Dualität der Bestandteile des Bastardkernes. *Planta* 18:751-785. 1933.

3. Love, R. M. Somatic variations of chromosome numbers in hybrid wheats. *Genetics* 23:517-522. 1938.

4. Yasui, K. Cytogenetici studies in artificially raised interspecific hybrids of *Papaver*. VI. The trigenomic hybrids of *Papaver*. *Cytologia*, Jubilee Volume, 1101-1112. 1937.

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LYSENKO PROGRESSES BACKWARDS

LYSENKO'S new book* begins with a brief outline of the history of the species concet as developed by Linnaeus, Lamarck, Darwin, and Timiriazev. The two last named authors are mildly reproved for having entertained a theory of "one-sided, flat evolutionism," which assumes that new species arise gradually from old ones, which consorts with the "reactionary pseudo-scientific doctrine of Malthus about intraspecific struggle." This one-sidedness and flatness were done away with by Michurin and by Stalin, who invented "creative Darwinism." The latter "visualizes the development not as a flat evolution, but as a birth in the interior of the old of its opposite new quality, which undergoes a gradual quantitative accumulation of its characteristics, and, in the process of the struggle with the old quality, forms itself into a new fundamentally distinct system of characteristics with its own distinctive law of existence." The clarity of this definition of creative Darwinism is matched by the following definition of species: "A species is a special qualitatively defined status of the living forms of matter. An important characteristic of the species of plants, animals, and microorganisms lies in definite intraspecific relationships among individuals. These intraspecific relationships are qualitatively distinct from the relationships among individuals of distinct species. Hence, the qualitative distinction between the intraspecific and the interspecific relationships is one of the most important criteria for distinguishing between species and varieties." The view that varieties are incipient species is a part of the flat evolutionism. It must be

*T. D. Lysenko, *New in the Science of Biological Species* (*Novoe v Nauke o Biologyicheskom Vide*). 30 pp. Selkhozgiz, Moscow, edition of 100,000 copies. Price 30 kopeks. Contains an article on "Species" written for the 2nd Edition of the "Great Soviet Encyclopedia," and a speech on "The Work of the Active Member of the Academy of Medical Sciences, U.S.S.R., O. B. Lepeshinskaia," read at a meeting of the Section of Biological Sciences of the Academy of Sciences of the U.S.S.R. in May of 1950. (1952).

replaced by the view that "species are links in the chain of living nature, or steps of the gradual historical development of the organic world." "The origin and development of new species depends upon such alterations of the metabolism in the process of the development of organisms which involve their species specificity."

If the reader is not profoundly impressed by the above philosophic foundations of "creative Darwinism" he surely can not fail to be so impressed by its experimental verifications. "In 1948 the experiments of V. K. Karapetjan disclosed that some winter sown plants of the hard 28-chromosome wheat *Triticum durum* became rather rapidly, in two-three generations, transformed into a different species, the soft 42-chromosome wheat, *Triticum vulgare*." This splendid success has stimulated even more ambitious undertakings. "In 1949, there was organized a search of seeds of rye in the ears of wheat grown on fields in the foothills of mountains, where the plantings of winter wheat often become contaminated with rye. The source of the contamination of wheat by rye in these localities was not known to science until a few years ago. The investigators V. K. Karapetjan, M. M. Jakubzimer, V. N. Gromachevsky, and also several other investigators, agronomists, and students have discovered isolated seeds of rye in the ears of hard as well as of soft species of wheat. In 1949 more than 200 such rye seeds were found. These seeds were sown at the Institute of Genetics of the Academy of Sciences of the U. S. S. R., on the experimental field of the V. I. Lenin All-Union Academy of Agriculture Sciences, and at the K. A. Timiriazev Moscow Agricultural Academy. . . . From the rye seeds developed in the ears of hard and of soft wheat there grew, with few exceptions, various but still typical rye plants. Only in a few instances the rye-like seeds gave rise to wheat plants."

The avalanche of discoveries proved hard to stop: "When the branched wheat *Triticum turgidum* was cultivated in the experimental plots of the V. I. Lenin All-Union Academy of Agricultural Sciences, and also in several other places, annually there was observed on these wheat plots contaminations by soft and hard wheats, by oats, by two-rowed and four-rowed barleys, and also by spring rye. All our observations have led us to the conclusion that the source of these contaminants is the branched wheat, *Triticum turgidum*, itself." To be sure, such contaminations were observed in the past, even as far as the first half of the nineteenth century. "But all such discoveries of one plant species in the fields of other species were, as a matter of principle, not considered by scientists as resulting from transformations of one species into another. Always there arose legitimate doubts. The possibility of the contamination being due to the often met with mechanical admixture of seeds could not be excluded. One could not be sure that the seeds sown did not contain some seeds of other species; that seeds of other species were not introduced on the field by water, wind, or by birds; that the contaminant seeds were not conserved for a long time in the soil, etc. . . . To the cases quoted above all the objections just enumerated do not apply. Indeed, scattered rye seeds found in wheat ears which grew for several generations under definite conditions could not be placed in these ears either by birds, or by men, or by any other means. These rye seeds have been produced by wheat plants and have developed in wheat ears."

Further achievements are confidently predicted: "The factual data so far obtained concerning species formation deal with the plant world only. Necessary factual data are still absent about species formation in the animal world. But one may feel certain that the development of the theory of Michurinist biology will in the near future give an opportunity to assemble factual data concerning zoological materials analogous to those concerning botanical ones." To Lysenko, the observations which he reports seem neither startling nor incredible. On the contrary, such things are just what a Michurinist biologist expects. Indeed, "when plants of a given species encounter conditions which are relatively unfavorable for a normal development of their species specificity, there occurs an enforced change, generation in the body of a given species of vestiges of another species, the specific formation of which is more suitable under new conditions of the external environment. The scattered individuals of the new species generated in the interior of the old one are better suited to the new conditions, they multiply rapidly and are capable of displacing the species in the interior of which they were generated." The presence of weeds is thus explained very simply: "Science knew for a long time that many species of weeds exist only in agronomic practice; in free nature these species are not only absent but they are even incapable of existing there. . . . Weed species are generated by some wild species as well as by cultivated species; thus this malignant pest the wild oat [*Avena barbata*] is generated by cultivated oats." "This explains why the wild progenitors of many cultivated plant species have not been found." One wonders at this point whether Lysenko ever heard about the possibility that the environment may, by means of natural selection, induce formation of adaptive genotypes from the genetic elements present in the populations of the parental species. Or is such an evolutionism too "flat" for him?

He next proceeds to incorporate the wonderful discovery of his fellow Michurinist, O. B. Lepeshinskaia, that cells arise from cell-less materials, into his theory. "Biologists who

stand on the Marxian theory of development clearly see the falsity of the postulate that plant and animal cells develop only from cells. . . . If cells arise only from cells, and even from similar cells, then how can an egg cell give rise in the developing organisms to various tissues with most different cells? Moreover, how is the egg cell itself formed? . . . It is absolutely clear to me that no theory of development is possible without acceptance of the view that cells arise from noncellular substances. As stated above, O. B. Lepeshinskaia has demonstrated experimentally that cells not only can arise but regularly do arise in any organism, and especially in the early developmental stages, from matter which has no cellular structure. This postulate and the experimental data obtained by O. B. Lepeshinskaia are basic for understanding many problems of the theory of individual organic development. No less important are the postulate and the experimental data of O. B. Lepeshinskaia for building a true theory of species formation."

"A large body of facts has been accumulated showing that rye may arise from wheat, and that different wheat species may give rise to rye. The same wheat species can generate barley. Rye can also generate wheat. Oats can generate wild oats [*Avena barbata*], etc. All depends upon the environment in which these plants develop. . . . Indeed, it is now completely demonstrated, and anyone interested can easily convince himself, that in the bodies of plant organisms of various species are generated and formed rudiments of bodies of individuals of other species. How does this happen? For example, can one imagine that a cell of the wheat plant becomes transformed into a rye cell? This I can not imagine. This is impossible. We visualize this thing as follows: In the body of a wheat plant, under the influence of proper conditions of life, there are generated granules of a rye body. But this generation does not occur by a transformation of the old into new, in this case, of wheat cells into rye cells, but by way of origination in the inner depths of the body of a given species, from a substance devoid of cellular structure, of granules of the body of another species. To start with, these granules also lack cellular structure, but they give rise later to cells and to rudiments of other species. This is what the works of O. B. Lepeshinskaia contribute toward the theory of species formation. The scientific postulates of O. B. Lepeshinskaia, together with other achievements of science, will form the basis of our developing Michurinian biology."

Lysenko's meteoric career in science has now lasted for almost a quarter of a century. It is most remarkable that during this time neither Lysenko nor any of his followers produced a single new idea, either a right or a wrong one. One might have thought that some original thinking would have occurred almost by accident. Lysenko began by rejection of all the findings of genetics, thus simply falling back to the level of knowledge which obtained in biology toward the close of the last century, which he found conveniently summarized for him in the popular writings of Timiriazev and of other Russian earlier Darwinists. But the grotesque idolatry of Timiriazev and Darwin proved also transient. With the rejection of Darwin's natural selection and of the cell theory, the level of biological knowledge reduces to somewhere back in the first half of the nineteenth century. Lysenko's belief in wheat suddenly generating rye and barley, and vice versa, is harder to date. Evolution through sudden origin of monstrosities was, of course, envisaged by Saint-Hilaire in 1830. But the "granules" which Lysenko imagines to be "generated" in some "depths of the body" sound very much like the kind of spontaneous generation which Spallanzani refuted as far back as 1775. It is impossible to tell from his writings whether Lysenko realizes all this. But it is revolting to see numerous competent biologists in the U. S. S. R. being forced to pay at least lip service to "Michurinist Biology" in their publications.

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Oh, Spinach!

SEVERAL correspondents call attention to a deplorable editorial lapse in the November-December issue of the *Journal of Heredity* page 299. *Senecio vulgaris* is described as "a spinach relative." Actually its relationship is more than remote, *Senecio* being a compositae and spinach (*Spinacia*) being a member of the Chenopodiaceae.

This blunder appears to have been occasioned by reliance on the telephone as a source of exact technical information. The Editor, who does not like the stuff, has ingested a very humble plate of the chenopod—not the composite.—R. C.

MAMMALIAN CHROMOSOMES IN VITRO

II. A Method for Spreading the Chromosomes of Cells in Tissue Culture

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CYTOLOGISTS have been greatly handicapped in the study of mammalian and avian chromosomes by the fact that most species possess large numbers of chromosomes which usually crowd on the metaphase plate and make counting and observation of individual chromosomes very difficult. Even the squash method which serves well in species containing low chromosome numbers does not give good results in most mammals and birds.

Quite recently, however, in his analysis of the effects of abnormal tonicity of saline on dividing chick osteoblasts *in vitro*, Hughes[6] pointed out the possibility of studying the chromosomes of higher animals by means of hypotonic solution treatment. In our laboratory a similar conclusion had been reached independently but accidentally, that hypotonic salt solutions swell human cells in tissue culture and disturb the spindles of the dividing cells. Consequently the prometaphasic and metaphasic chromosomes are widely spread apart and are thus available for critical studies. The result of observations on human chromosomes was reported by one of us (Hsu[3]).

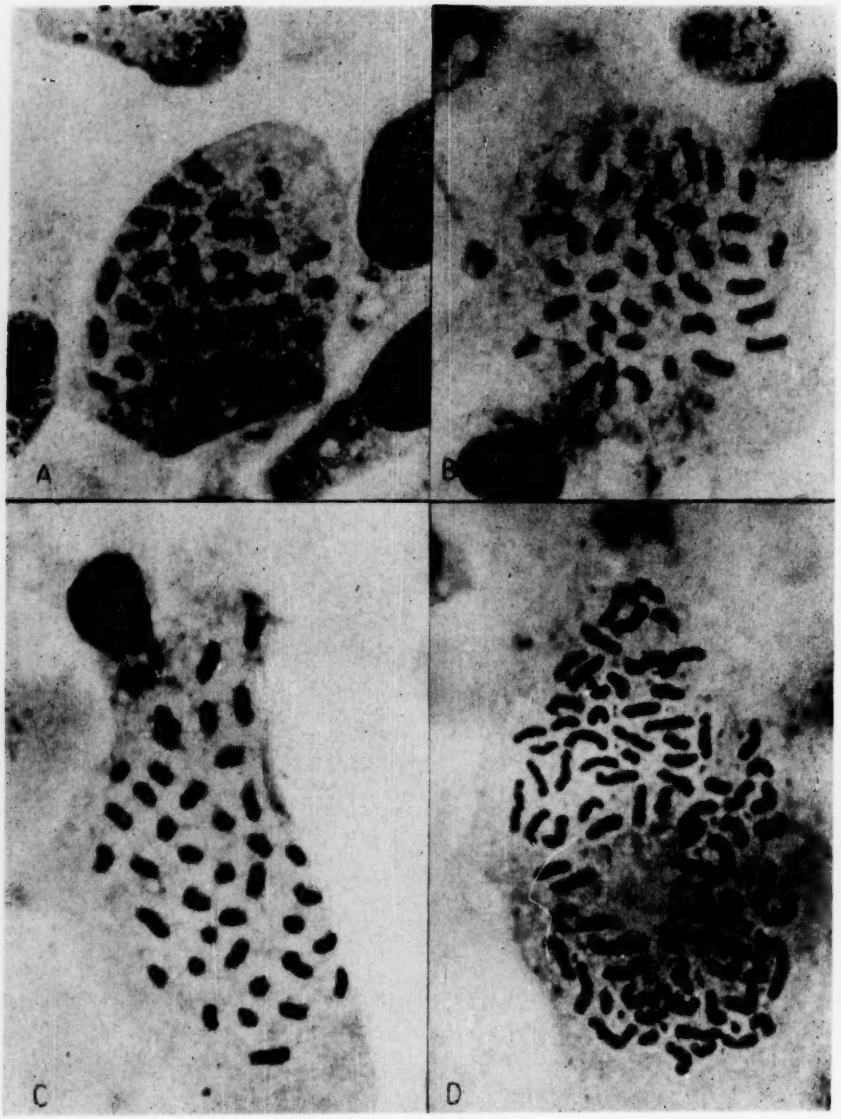
Several species of laboratory animals have since been examined with this method and the results obtained give promise of its wide applicability on mammalian and avian materials. The present report is concerned with some illustrations of the chromosome plates of the several species studied with tissue culture materials treated with the hypotonic saline prior to fixation. Suggestions for employing this method in karyologic investigations are also given.

Figure 10 *A* and *B* represent cells from embryonic heart of the mouse (*Mus musculus*, albino) cultivated at 37°C for three days, each exhibiting 40 rod-shaped chromosomes, the diploid number of this species. In Figure 10 *C* two chromosomes are united in a cross-shaped figure, suggestive of a spontaneous chromatid exchange. This suggests that the effect of radiation and drugs on mammalian chromosomes might be re-examined profitably by means of this technique. Figure 10 *D* represents a tetraploid cell from a lung culture of the same species in which 80 chromosomes can be counted; the origin of the numerous fragments in this figure is not known for none of the other cells contained such fragments.

Like *Mus musculus*, the guinea pig (*Cavia cobaya*) is an excellent material for chromosomal investigations, although the diploid number is as high as 64. Figure 11 *A-D* illustrates examples of cells selected from the lung of guinea pig embryos in culture for five days. Figure 11 *A* represents a cell in which almost all the chromosomes are lying on one plane. It is of interest to note from this figure that the two longest chromosomes have knob-like second arms. In Figure 11 *C*, the condensation of the chromosomes is more pronounced probably because the division stage is approaching anaphase. The chromatids are already separate in Figure 11 *C* and are more so in Figure 11 *D*. Due to the inhibition of the spindle apparatus at metaphase or even earlier stages resulting from treatment with hypotonic solution, chromatids reaching anaphase separate autonomously but fail to move to the poles. This behavior is quite analogous to the action of colchicine on plant cells.

Two swollen metaphases of the cotton rat (*Sigmodon hispidus taxianus*) are shown in Figure 12 *A* and *B*, both taken from lung cultures of a young adult, fixed in Bouin's fluid.

*Aided by a grant from the American Cancer Society CP-12E. Dr. Hsu is a Damon Runyon Fellow. Acknowledgement is made to Mr. George Lefeber for his assistance in photographic work.

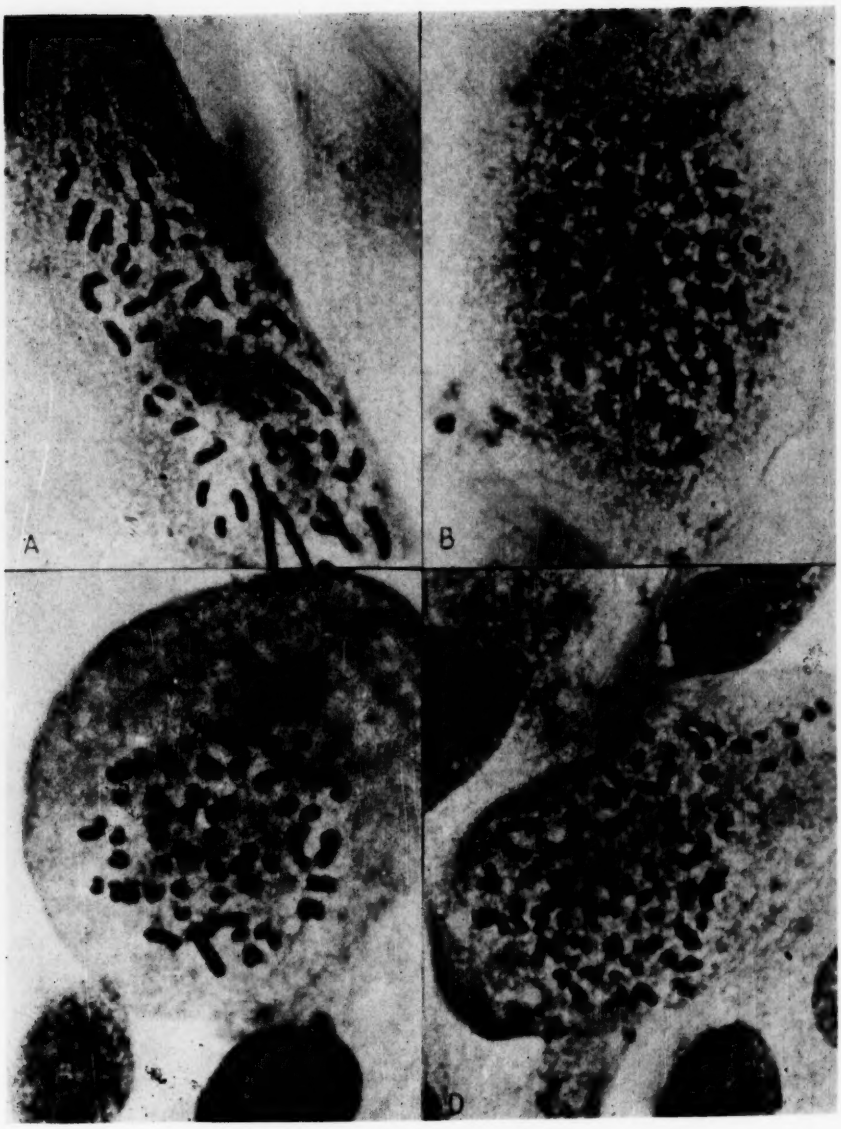


HEART AND LUNG CULTURE OF THE ALBINO MOUSE

Figure 10

Metaphase from hanging drop cultures of embryonic tissues, pre-treated with hypotonic saline (30 percent full formula Gey's and 70 percent Gey's minus NaCl) for 20 minutes on the third day, fixed in Helly Zenker's and stained with Delafield haematoxylin.

A and *B* show diploid heart cells. *C*—Heart cell, showing chromatid exchange between two chromosomes. *D*—Tetraploid lung cell with fragments. 1450×.



EMBRYONIC LUNG CULTURE OF THE GUINEA PIG

Figure 11

Cells from roller tube cultures of embryonic lungs, pre-treated with hypotonic saline (20 percent full formula Gey's and 80 percent Gey's minus NaCl) for 20 minutes on the fifth day, fixed in Helly Zenker's and stained with Delafield haematoxylin.

A-C shows Metaphases. *D* shows anaphase in the absence of the spindle. 1450×.

The chromosomes are dispersed at several focal levels and are thus not suitable for photographic records. Bouin fixation seems to cause this dispersion while Helly Zenker's results in a rather flat plane, since some sets of cultures of other animals fixed in Bouin's have a similar defect. Also, Bouin's fixative produces a coarse structure of the cytoplasm, presumably due to the action of the acids in this fluid.

Cancer cells react to hypotonic saline in the same manner as corresponding non-malignant elements. In a set of cultures of a human malignant sarcoma (Patient E. A., Path. #S-35250, John Sealy Hospital), the chromosome number was found to have wide variation but a number of them could still be counted. Figure 12 *C* and *D* represent metaphases of two heteroploid cells from this neoplastic tissue.

The domestic dog (*Canis familiaris*) is known to have 78 chromosomes as its diploid number, probably one of the highest among Mammalia (cf., Makino[6]). In slides made from tissue cultures of embryonic lung and heart our counts confirm that 78 is the correct number. Two metaphases are illustrated in Figure 13 *A* and *B*.

Hypotonic saline also swells to some extent the late prophase and anaphase. Figure 13 *C* shows a cell in late prophase and Figure 13 *D* at anaphase, both from lung cultures of dog embryos. Critical counts of the two daughter chromosome groups during anaphase are indeed needed to throw some light on the problem of non-disjunction because somatic aneuploidy has been repeatedly reported and in our materials this phenomenon also exists.

Makino and Nishimura[7] employ a water pretreatment method for testis squashes of various animals and claim success with this method for chromosome studies. It has been pointed out by Hughes[5] and by Crawford and Barer,[1] however, that pure water or even highly hypotonic solution damages the cytoplasm and eventually bursts the cells. In this laboratory, several tissue fragments not having been cultured were tested with hypotonic solution treatment and the resulting metaphase figures in aceto-orcein squashes showed some improvement as compared with the untreated series. Unfortunately this method, although much simpler to perform than the tissue culture technique, has a number of disadvantages. First, the swelling of the cells, due to the thickness of the tissue block, is never as complete as that in tissue culture where the outgrowth zone is usually but one cell thick. Moreover, under these conditions the cells are usually markedly flattened.[2] Consequently, the chromosome plates are much more widely spread in the *in vitro* material. Second, in order to get large numbers of dividing cells, only those rapidly growing tissues can be used for the squash method whereas in tissue culture some adult organs, notably lung, bone marrow and many fibroblastic tissues grow vigorously in test tubes. Third, due to the swelling of the cells by the treatment, subsequent squashing breaks many cells and scatters the chromosomes over the slides, thus making the counting work more inaccurate. Finally, for the squash preparations only the acetic stains and the Feulgen reagent can be used while with the *in vitro* materials nearly all the standard fixation and staining procedures for tissue cultures can be followed, including phase contrast microscopy of the living cells. Nevertheless, these points do not nullify the value of the Makino method since, in studies of meiosis, there is still no satisfactory method of culturing the spermatocytes or the oocytes.

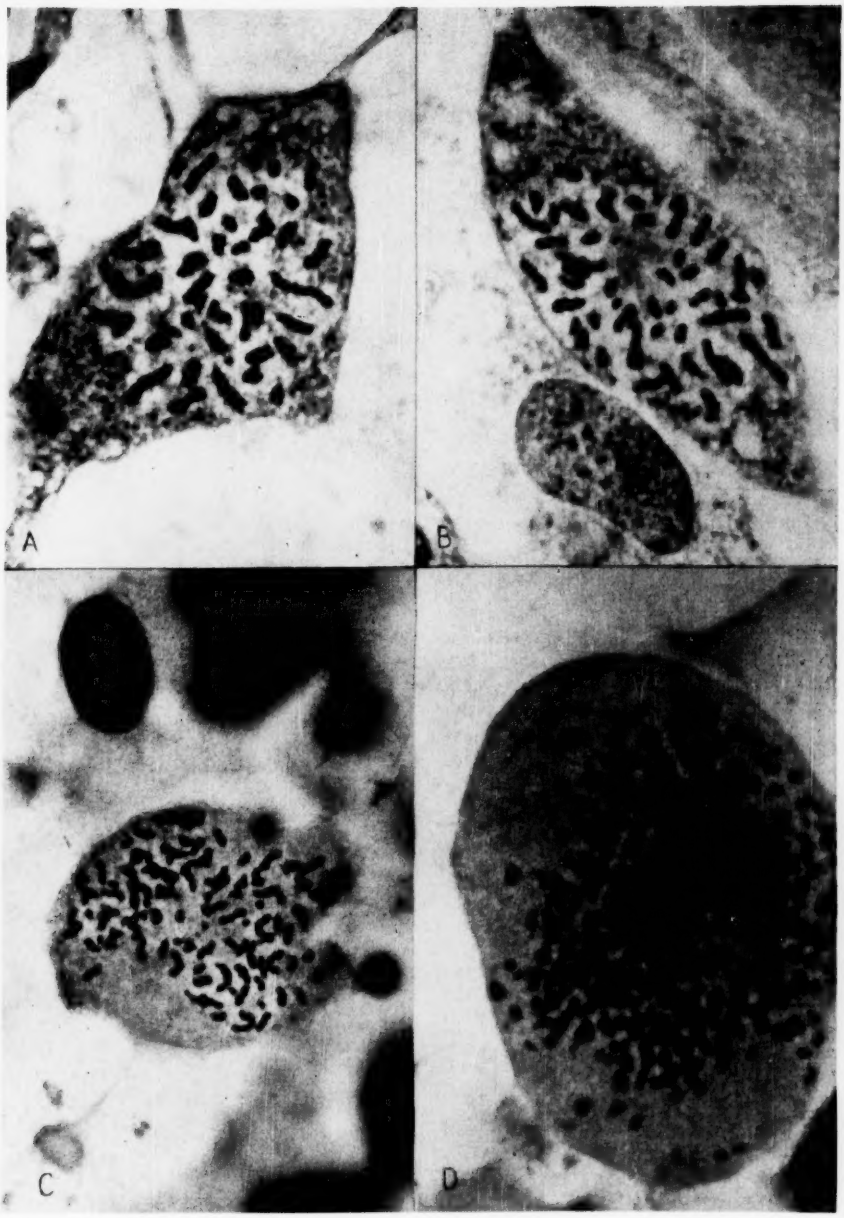
The choice of methods for tissue culture depends upon the age of the tissue donors. A simple plasma clot (equal parts of heparinized rooster plasma and chick embryonic extract) in hanging drop slides is adequate for early embryonic tissues, whereas a fluid-phase medium in roller tubes is necessary for late embryonic and adult tissues (cf., Pomerat[3]). While the roller tube method is well-known, we have found special advantage in embedding five explants on each of two 12 × 50 mm #1 thinness cover slips which are placed back to back into a standard 16× 150 mm Pyrex test tube. When the medium has become firmly clotted, 2 ml of a fluid nutrient consisting of 50 percent human ascitic fluid of malignant origin, 45 percent of Gey's balanced salt solution and 5 percent of chick embryonic extract are added. The inclusion of 1000 units per ml of penicillin G aids in the reduction of chance contamination. Recently, however, it has been found that a combination of 100 micrograms each of bacitracin and neomycin per ml is even more efficaceous particularly in the management of surgical material. The concentration of the antibiotics employed appears to be well removed from the zone of minimal injury as revealed by toxicity tests.[4]

To produce the swelling effect, a warmed hypotonic solution is prepared by mixing 20 to 30 parts of normal Gey's balanced salt solution with 80 to 70 parts of a similar mixture but containing no sodium chloride. Living cultures are immersed in this solution for 20 to 30 minutes and then fixed. Helly Zenker's fixation for one hour is followed by either Delafield haematoxylin or haematoxylin-eosin-azure which gives satisfactory preparations as has been demonstrated in the figures.

Summary

Illustrations of chromosome plates resulting from pre-fixation treatment of cells in tissue culture with hypotonic solution are shown for the mouse, guinea pig, cotton rat, dog, and for a human tumor.

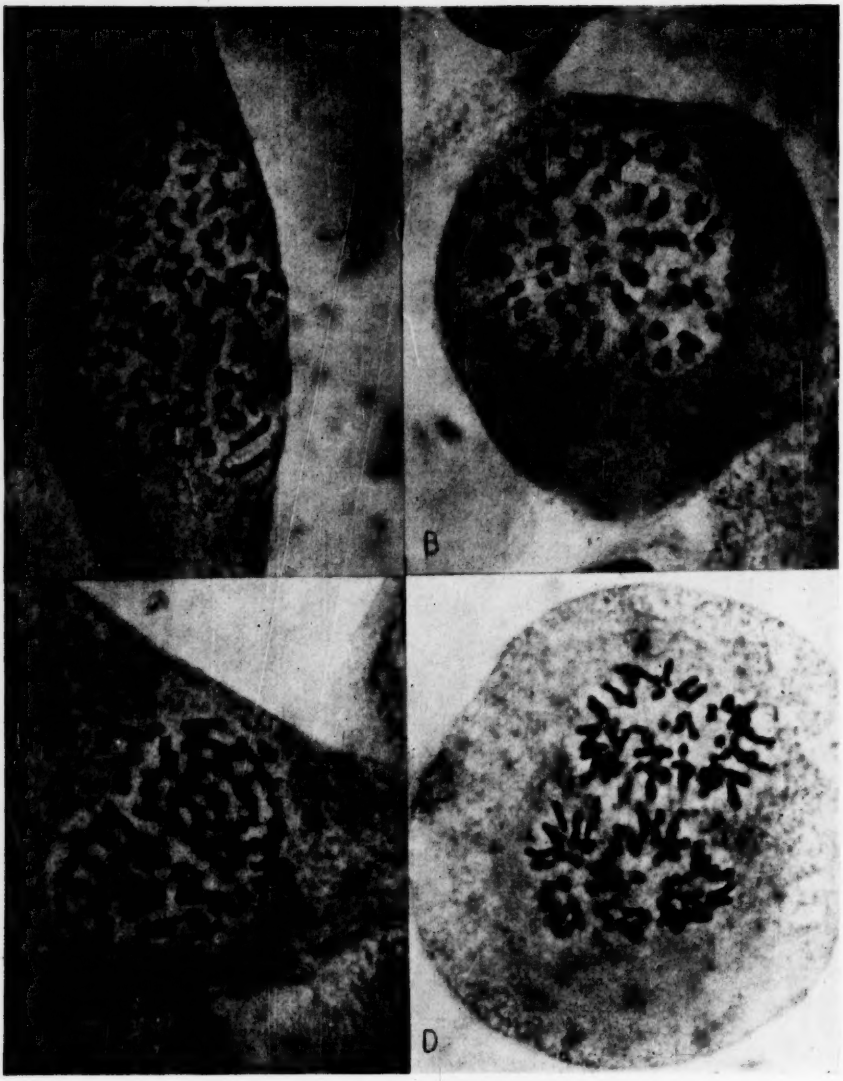
A method is described which involves tissue cultures of simple hanging drop slides and the roller tube technique. A balanced salt solution (Gey's) containing no sodium chloride is



CULTURES OF THE COTTON RAT AND A HUMAN LIPOSARCOMA

Figure 12

A and *B*—the cotton rat. Metaphases from roller tube cultures of adult lungs, pre-treated with hypotonic saline (20 percent full formula Gey's and 80 percent Gey's minus NaCl) for 20 minutes on the fifth day, fixed in Bouin's and stained with Delafield haematoxylin. *C* and *D*—Human sarcoma. Metaphases from roller tube cultures, pre-treated with hypotonic saline (30 percent full formula Gey's and 70 percent Gey's minus NaCl) for 20 minutes on the sixth day, fixed in Helly Zenker's and stained with Delafield haematoxylin. 1450×.



EMBRYONIC LUNG CELLS OF THE DOG

Figure 13

Cells from roller tube cultures of embryonic lungs, pre-treated with hypotonic saline (20 percent full formula Gey's and 80 percent Gey's minus NaCl) for 30 minutes on the fourth day, fixed in Helly Zenker's and stained with Delafield haematoxylin. *A* and *B*—Metaphases; *C*—Prophases; *D*—Anaphase.

mixed at a proportion of 70 to 80 parts with 30 to 20 parts of the regular Gey's solution and is applied to the cells in culture for 20 minutes before fixation. Helly Zenker's fixation followed by Delafield haematoxylin or haematoxylin-eosin-azure stain is recommended.

The method gives great promise in studying mammalian, avian and other animal cells with large numbers of chromosomes. Special attention is directed to the opportunity of studying malignant elements with this method as well as the effects of radiation and carcinostatic chemicals.

Literature Cited

1. Crawford, G. N. C. and R. Barer. The action of formaldehyde on living cells as studied by phase contrast microscopy. *Quart. Jour. Micro. Sci.* 92:403. 1951.

2. Hsu, T. C. Tissue culture studied on human skin. III. Some cytological features of the outgrowth of epithelial cells. *Texas Rep. Biol. and Med.* 10:336. 1952.

3. ———. Mammalian chromosomes *in vitro*. I. The karyotype of man. *Jour. Hered.* 43:167. 1952.

4. Hu, F. N., C. S. Livingood, P. Johnson and C. M. Pomerat. Tissue culture studies on human skin. IV. The comparative toxic effects of antibiotics on tissue culture explants of human skin and embryonic chick spleen. *Jour. Invest. Derm.* (in press)

5. Hughes, A. Some effects of abnormal tonicity on dividing cells in chick tissue cultures. *Quart. Jour. Micro. Sci.* 93: 207. 1952.

6. Makino, S. An Atlas of the Chromosome Numbers in Animals. Iowa State College Press, Ames. 1951.

7. Makino, S. and I. Nishimura. Water-pretreatment squash technic. *Stain Tech.* 27: 1. 1952.

8. Pomerat, C. M. Methods in Medical Research, Vol. IV. Year Book Publishers, Chicago, pp. 198-291. 1951.

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The Twenty-Fourth Annual Meeting of The Genetics Society of Japan

THE twenty-fourth annual meeting of the Genetics Society of Japan was held at Niigata from October 8 to 10, 1952. Dr. Hitoshi Kihara presided. One hundred and forty geneticsts participated in the program. (Incidentally, Dr. Kihara is now visiting genetics departments and laboratories of several major universities in this country.)

The first day of the meeting was devoted to general session invitation and award lectures. These included an award lecture entitled "Human Population Genetics" (T. Komai) and "Chromosome Structure as Observed by the Electron Microscope" (G. Yasuzumi).

During the next two days, one hundred and sixty-four papers were delivered. They covered an extremely wide range of genetical and cytological investigations involving a surprisingly large number of genera of both plants and animals. To facilitate the presentation of so many papers, the meeting was divided into botanical and zoological sessions which ran simultaneously.

Eighty-one papers dealing with plant genetics and cytology were presented. They included discussions of polyploidy, mutable genes, sex, incompatability, sterility, biochemical mutants, chemical and radiation effects on cells and chromosomes, heterosis, and numerous cytological and cytogenetical studies. These involved organisms ranging from forest trees to fungi and bacteria, and included, among others wheat, maize, barley, rice, hops, sugar beets, watermelon, as well as representatives of the following genera: *Nicotiana, Tradescantia, Trillium.*

The subject matter of eighty-three papers delivered on animal genetics and cytology included, besides most of the topics listed above, cancer, population studies, chromosome chemistry, gene action, and phenocopies. The organisms employed in these studies ranged from man to tripanosomes and bacteria, included among others, *Drosophilla*, silk worm, mice, fish, salamander, locusts, snails, earthworms, ladybird beetles, and spiders.

All that this brief summary allows is an indication of the wide variety of genetical and cytological discussions at this meeting. The program of the meeting has been translated and is available to those who are interested in more details of the subject matter covered and the names and locations of the Japanese geneticists engaged in the numerous investigations. The translation is in mimeographed form and can be obtained upon request from Dr. R. A. Nilan, Department of Agronomy, State College of Washington, Pullman.

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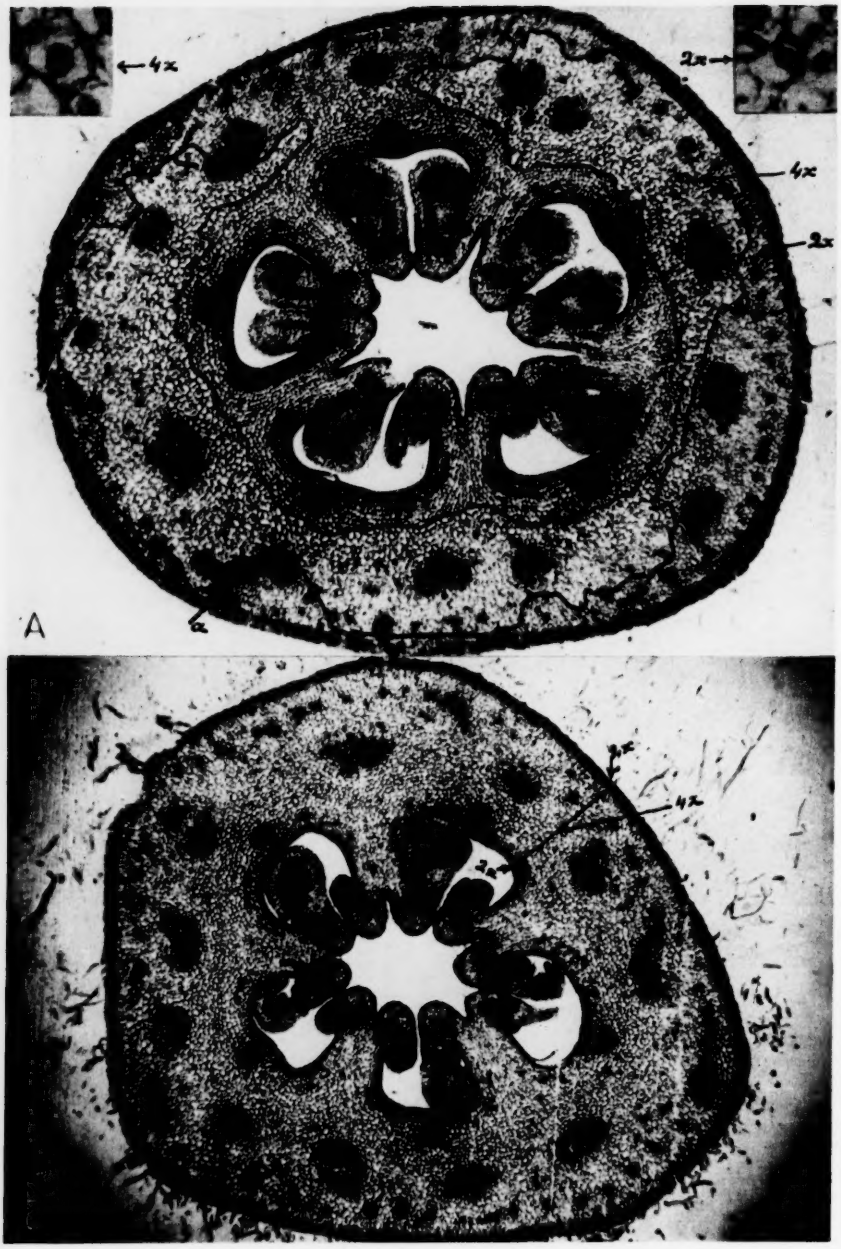

OVULAR LOCULES

Figure 14

A—Transverse section through ovular locules in an ovary at the pink stage of flower development. The portion enclosed by an ink line is 4*x* and the tissue in other areas is 2*x*. (See text for the two chromosome plates above *A* and for other details.) *B*—A similar section of an ovary of the same age as that of *A*. The internal tissue is almost entirely 4*x*. *A* is slightly more magnified than *B*.

THE PATTERN OF TETRAPLOIDY

In the Flower and Fruit of a Cytochimeral Apple

HAIG DERMEN*

IN a preceding article[8] a study of the relationship between the histogenic† cell layers of the shoot apex and the tissues of the leaves and stem in the apple was reported. The study was made of diplo-tetraploid chimeral plants in which the ploidy of tissues in various regions in the leaf and the stem was traced into the shoot apex, thus establishing directly certain relationships between the individual histogenic layers in the shoot apex and the origin of tissues in the leaves and the stem. A similar study of the relationship between the tissues and the histogenic layers of various flower parts of the apple, especially of the ovary‡, and of the fruit is reported herein. In addition, evidence is presented in support of the theory of the "appendicular" origin of the fruit as opposed to the "receptacular" theory. Cytochimeral material,§ such as studied here, is unique and peculiarly adapted to aid in the analysis of certain botanical problems, such as time of vascular tissue development in leaves and flower parts, and problems of morphogenetic nature.

Blaser and Einset[2] have presented a series of diagrams in support of their observations on the cytochimeral development of the tissues in all parts of the flower. They have also discussed the question of whether the fruit of the apple is morphogenetically of receptacular or appendicular origin and have arrived at some conclusions in support of the appendicular theory. Satina and Blakeslee[16, 17] and Satina[14, 15] have made very exhaustive studies of the ontogeny of tissues in the flower parts of cytochimeral plants of *Datura stramonium*. Dermen[5] has made some observations concerning ontogeny of the tissues in the ovary and ovules of the cranberry. The references mentioned above seem to be the only ones dealing with studies of ontogeny of tissues of the flowers based on observations of cytochimeral plants.

Materials and Methods

The present study was made from material of a diploid-diploid-tetraploid (2-2-4) chimeral Winesap apple variety[8]. Flowers were studied at various stages of development: (1) tight bud stage, in the first week of April, 1951; (2) just before buds are loosening; and (3) at pink bud stage. Fruits were studied (1) at about petal fall; and (2) when they measured about 7mm. in diameter (latter part of May). The total number of individual flowers and fruits studied was about 50. From five (five sepals, five petals, etc.) to 20 (10 ovules, 20 stamens) times more flower parts than actual number of flowers and fruits were examined.

As in the leaves[8] the determination of ploidy of cells in various areas of tissues in all the flower parts was based collectively on chromosome plates, nuclear size, and cell size. In these tissues a definite relationship between ploidy and nuclear size was found to exist between cells in adjacent areas in a given tissue. The relationship between ploidy and cell size in such areas was less apparent because in a meristematic tissue where there is active cell division, some daughter cells have not become fully enlarged. Since the cells

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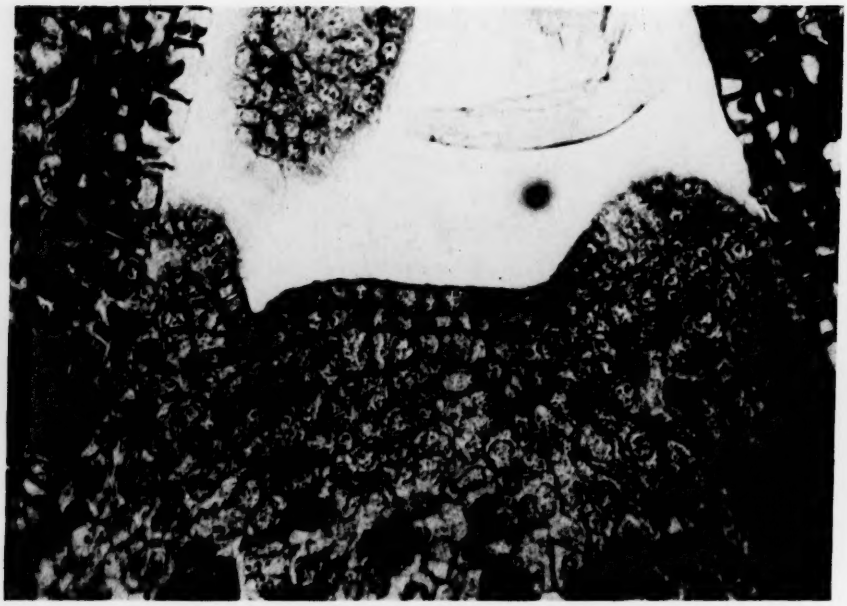
†The term "histogenic" has been applied to designate, in a restricted sense, the apical layers in cranberry [4,5] and in apple[8]. In a similar sense, and more as a matter of convenience, this term is used here.

‡In this article "ovary" refers to the lower, fleshy part of the apple flower, which matures into the fruit. In the apple, ovary and fruit being morphologically and histologically the same, they are referred to interchangeably in the text.

§Cytochimera refers to that chimeral condition of plants where tissues or regions in the plant system differ in their chromosome numbers.



SHOOT APEX OF A CHIMERAL APPLE

Figure 15

Longitudinal section of the shoot apex of a *2-2-4* chimeral apple. In the central region above the ink line are L-I and L-II which are *2x* and below the line is L-III which is *4x*.

and nuclei in a given tissue do not lie on one plane, some are sectioned through their median planes, others through their narrower parts. Therefore, in the sections illustrated, the cells and nuclei of a tissue show a greater variation in size than is actually so. In the illustrations, except in Figure 14*B*, the demarcation between diploid and tetraploid areas has been indicated by an ink line. Because of low magnification, *2x* and *4x* areas in Figure 14*B* are indicated differently. For complete accuracy the boundary line between diploid and tetrapoid areas was determined by examining the sections under oil immersion and then the ink line was drawn step by step along the cell walls separating adjacent areas differing in ploidy. In meristematic tissues there was no difficulty in determining that diploid chromosome plates were found only in areas where nuclei were small and tetraploid plates in areas where nuclei were large.

Observations

Cytohistological determination in tissues of flower parts. The cytochimeral constitution of a plant is determined primarily and most accurately from a study of the shoot apex. Figure 15 shows a longitudinal section of the apical region of the shoot of a *2-2-4* chimeral apple. The stem apex, the "apical dome," in Figure 15 is the central region above the ink line. The two cell layers in the dome region above the line are L-I (the first apical layer) and L-II (the second layer), and the layer below the line is L-III (the third layer). The cells in L-I and L-II are small and are *2x*. The cells in L-III and in the lower tissues derived from it are larger and are *4x*. The elevated body of tissue at the right of the shoot apex in Figure 15 is a section through a leaf primordium. A *4x* plate (indicated by an arrow) is present in L-III in Figure 15. Other *4x* plates inside the ink line are indicated by crossmarks. Another crossmark outside the ink line in the leaf primordium at the right indicates a *2x* plate.

In the *2-2-4* chimeral tree, flowers taken from branches with slender growth were entirely diploid, while those from thicker branches contained tetraploidy to a varying extent. The tetraploidy in the flowers was entirely confined to the ovary and was absent in ovules, styles (stigma included with style), stamens, petals, and sepals. The extent of tetraploidy in the bud scales was similar to that in the leaf[8] and the ovary.

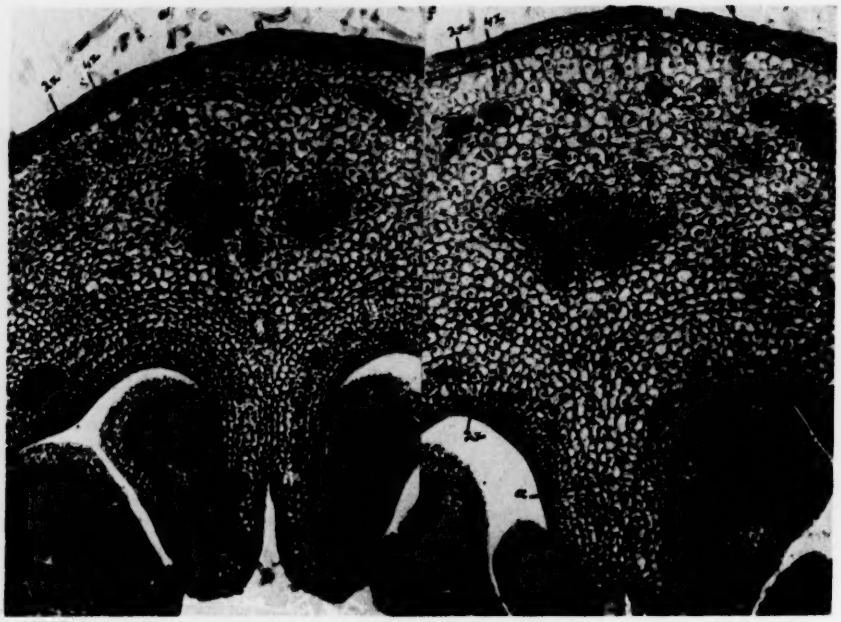
LONGITUDINAL SECTION OF THE OVARY

Figure 16

A shows the longitudinal section through the middle of the ovary portion of a flower at a stage just before the flower cluster becomes loose. The ink line delimits the 4*x* tissue marked by a long arrow at the left of the figure. The part outside the line, including all flower parts above the ovary is 2*x* marked by a short arrow also at the left of the figure. In the portion at the right a vein extends from the lower part of the flower into the base of a stamen; the ink line cuts across the vein. Upper part of the vein (marked *d*) is 2*x* and the lower part (marked *c*) is 4*x*. *B* shows the same area as in *A*, in which the vein is in part 2*x* and in part 4*x*, but from an adjacent section, showing the distinction between the 2*x* and 4*x* portions more clearly because of the contrast in nuclear size. *C*—shows the same area as shown in *B*, but at a higher magnification. The larger nuclei inside the ink line in both the vein cells and the smaller nuclei in the cells in the upper portion of the vein and the parenchyma tissue are seen more clearly.

Tetraploid and diploid areas in the ovary, just before loose bud-cluster stage of flowers, are shown in longisection in Figure 16*A*. The area within the ink line represents the tetraploid portion of the tissue in the ovary; (indicated by an arrow marked 4*x*) it is confined almost entirely to the ovary walls and basal part of the ovary. Elsewhere in the ovary and other flower parts the tissues are diploid (indicated by an arrow marked 2*x*). At the very base of the ovarian cavity in some flowers, tetraploidy was present in the tissue beginning at the fourth row of cells from the inner surface; in others tetraploidy in that area was found farther in (Figure 16*A*, at point *a*).

There was no ontogenetic relationship between ploidy and the conductive tissue system in any flower part. At the right in Figure 16*A*, a vein in the ovary wall extends from the lower portion of the ovary into the base of a stamen filament (at point *b*). Part of the vein tissue and the part of the parenchymatous tissue surrounding it are 4*x* (at area *c*), whereas the upper portion of the same vein with the surrounding parenchymatous tissue is 2*x* (at area *d*). Enlarged illustrations of the area where the ploidy in the vein changes from 4*x* to 2*x* are shown in Figure 16*B* and *C* (marked by arrows). The difference in ploidy in the two portions of the vein was determined mostly from nuclear size and chromosome plates and seldom from cell size. The area shown in Figure 16*B* is from a serial section following the one shown in Figure 16*A*. In



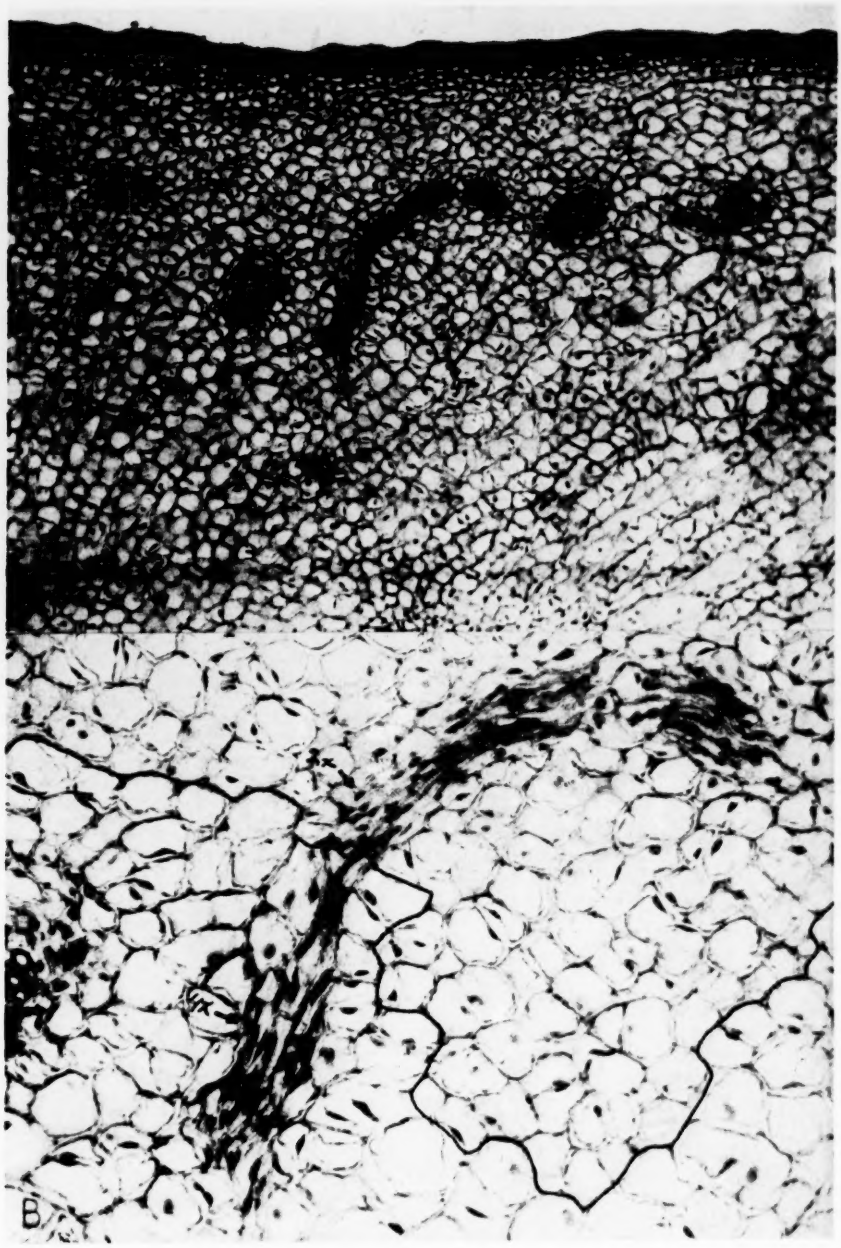
A SECTOR OF THE OVARY

Figure 17

A—A sector of the ovary section shown in Figure 14 *A*. *B*—A sector of the ovary shown in Figure 14 *B*. This material is similar to that shown in Figure 14, but is more highly magnified.

Figure 16*B* the contrast between the diploid and tetraploid portions in the vein is clearer than in Figure 16*A*. The differences in size of nuclei and also of the parenchymatous cells between the diploid and tetraploid portions are more conspicuous in Figure 16*B*. Because Figures 16*A* and 16*B* were prepared from two different sections, the ink lines do not coincide in the two figures. Figure 16*C* shows an enlarged view of the part in Figure 16*B* where the ink line separates the diploid and tetraploid areas. Small arrows in Figure 16*C* point to mitotic figures. It is noticeable in the higher magnifications (Figures 16*B*, *C*, 17 and 18) that the cells in the areas marked as tetraploid are, when viewed as a whole, larger than those in the adjoining diploid areas.

Figure 14*A* and *B* shows transections of two ovaries (at pink bud stage) at the ovular level. The ink line in Figure 14*A* encloses a central area that is tetraploid; the diploid areas are mostly in the inner and the outer portions of the ovary. At one point in the upper area of the section diploidy extends from the outer epidermis to the inner epidermis. In other parts of the section, some tetraploid areas are extensive and in other areas tetraploidy occurs in narrower strips, thus making a very asymmetrical pattern in the ovary. Irrespective of ploidy in these ovaries the number of cells, from the inner epidermis to the outer, varied in the different sectors of the ovaries. At the upper corners of Figure 14*A* are inserted two small figures. The one on the left represents a 4*x* chromosome plate and shows also the large size of the 4*x* cell. The location of this cell was in the upper left area of the ovary in Figure 14*A* indicated by a crossmark. The figure in the upper right corner shows a 2*x* chromosome plate in a smaller cell than that with the 4*x* chromosome plate. The location of this cell was in the upper right portion of the ovary indicated by another crossmark. Both chromosome plates were photographed at a higher magnification than Figure 14*A* and *B* from a serial section adjoining that shown in Figure 14*A*. In the ovary shown in Figure 14*B* all the tissue with the exception of about two or three rows of cells next to the outer and to the inner epidermis was tetraploid (marked 4*x*). Tetraploidy in this ovary extended vary-



OUTER PORTION OF WINESAP FRUIT

Figure 18

A—Transection of the outer portion of a Winesap fruit that measured about 7 mm. in diameter. The tissue is 4*x* below the ink line and 2*x* above it, as indicated by arrows. Near the center of the figure the line cuts across a vein; the upper part being 2*x* and the lower part, 4*x*. *B*—The long vein shown in *A* magnified further to show the large nuclei, spindle shaped, in the 4*x* part of the vein below the ink line and the small nuclei in the 2*x* part above the line. Arrows point to groups of nuclei in the two portions; small in size (2*x*) in the upper portion and large (4*x*) in the lower portion.

ing distances into the placental tissue (ovule bearing ridges, Figure 17*B*), but not into the stalks of the ovules.

An enlarged portion from each of the two ovaries is shown in Figure 17. Figure 17*A* is a section from the upper right portion of the ovary in Figure 14*A*. The enlargement shows more clearly the contrast in cell size between the tetraploid and the diploid areas, demarcated by an ink line. It is quite obvious that in these figures the cells and the nuclei in the tetraploid area are on the whole larger than those in the diploid area, and that tetraploidy is not confined to any particular tissue system either parenchymatous or conductive. Tetraploidy in this material is found at a considerable distance from the placental tissue protruding into the ovarian cavity and from the ovules. The enlarged area shown in Figure 17*B* is a sector from the top portion of the section in Figure 14*B*. Diploidy in the outer portion of this ovary (Figure 17*B*) is confined primarily to three rows of cells; in the inner portion at the ovular locules, diploidy is also confined for the most part to three rows of cells (at the region marked 2*x*). In the rest of the fruit the tissue is tetraploid. Tetraploidy in the ovary extends into the placental tissue (indicated by arrow *a*) and is quite close to the ovules, but never was found to extend into the ovules or even into the funicles.

In the lower left part of Figure 14*A*, a portion of a vein (dark body of tissue marked by arrow *a*) is in the tetraploid area and the rest of it is in the diploid area. A similar instance was observed in a section of a young fruit (at the stage of petal fall), a small sector of which is shown in Figure 18*A*. In this figure also a portion of a vein is in the diploid area (above the ink line at the upper center in Figure 18*A*) and the remaining portion is in the tetraploid area. Figure 18*B* is an enlargement of the middle vein in Figure 18*A*. Here the elongated, large nuclei (marked 4*x*) in the lower portion of the vein below the ink line are in the tetraploid part and the small nuclei (marked 2*x*) are in the diploid part, above the line. The pattern of tetraploidy throughout the young fruit is asymmetrical like that in the ovary in Figure 14*A*.

Discussion

Satina and Blakeslee[16,17] and Satina[14,15] have studied the ontogeny of tissues in flower parts of colchicine-induced cytochimeral plants of *Datura*. They have found that all three histogenic layers (L-I, L-II, and L-III) take part in the development of all flower parts: sepal, petal, stamen, carpel, and style. It appears from their report that only in the ovule L-III fails to extend into the main part, the nucellus; it seems to enter only the funicle. In *Datura* the contribution from L-III in the development of flower tissues appears to be similar to that reported occurring in the histogenesis of the leaves.[16]

A situation parallel to that occurring in *Datura* has been reported in the apple by Blaser and Einset.[2] They have presented the ontogenetic processes in flower parts in cytochimeral apples with diagrams. It appears from their statements and the diagrams in their article that in a 2-2-4 chimeral type plant of the Northern Spy variety the major veins, if not the minor ones as well, in the flower parts, such as a sepal, petal, stamen, and ovary, are tetraploid. They have reported that in the 2-2-2-4 chimeral Rome Beauty, tetraploidy is confined to the conductive tissue in the ovary and sepals and was absent in the petals and stamens. In the ovary of Rome Beauty flower they have reported "cords of tetraploid tissue enclosed by diploid cells."

In the present study of the flower of the 2-2-4 chimeral Winesap, no trace of tetraploidy was found in the sepals, petals, stamens, styles, and ovules. Tetraploidy was present in bud scales, and its extent throughout the scales was similar to that in the leaf.[8] In the ovary of some flowers almost all the internal tissue was tetraploid (Figures 14 *B* and 17 *B*), diploidy being confined to the epidermis and usually to only two rows of cells next to the outer epidermis and to the inner epidermis in the ovular locules. In the ovary of most of the flowers tetraploidy made an irregular pattern in the inner tissue (Figures 14 *A* and 17 *A*). This suggests that the variable and irregular shape, frequently characteristic of mature fruits of 2-2-4 chimeral apple varieties,[7] may be directly associated with irregularity of internal tetraploid tissue (See Figure 14 *A*). It is significant that no fruits of normal plants ever show the degree of asymmetry found in the fruits of the type of chimeral plants discussed here. In this connection may be mentioned the case of asymmetry of fruit shape

in an apple sport, named "Sweet and Sour".[6] The asymmetry in this fruit was attributed to a difference in the amount of tissues derived from L-II and L-III. The cytohistological evidence presented here (Figure 14 *A*) may be offered in support of the suggestion as to the basis of the asymmetry of fruit shape in the "Sweet and Sour" variety.

Conclusions as to whether the fruit of the apple is of "receptacular" origin[9, 10, 11, 13, 18] or, of "appendicular" origin[2, 12] have been based on anatomical and general morphological studies. Evidence from the present histogenetic study from the cytochimeral material seems to support the appendicular theory.

In the stem of the *2-2-4* chimeral apples[8] tetraploidy is usually found in the entire cortical tissue (except in the hypodermal region) and in the stele including the pith. In the *2-2-2-4* chimeral stem of McIntosh apple[8], just as in the *2-2-2-4* chimeral Rome Beauty studied by Blaser and Einset[1], about half of the cortex internally and all the rest of the internal tissues are tetraploid. In the leaves of these chimeral plants the pattern of the tetraploid zone is irregular[8]. Therefore, on the basis of the extent or pattern of tetraploidy in the ovary—now we many speak of it as the fruit—some internally completely tetraploid fruits (Figure 14*B*) may be said to resemble the stem. If the fruit, however, is considered to be like the stem, then tetraploidy should always be present in the fruits of these plants, since the pith in the stem in these chimeral plants is always tetraploid. As in the stem, the fruit would be the last area from which tetraploidy should disappear, indicating truly an ontogenetic relationship between an inner histogenic cell layer of the shoot apex and a pith tissue in the fruit. It was shown in Figure 14*A* that in one part of the ovary the tissue extending from the outer epidermis to the inner epidermis was diploid and in other parts narrower strips were tetraploid. The tetraploidy in other regions of the ovary covered wider areas. In Figure 14*A*, the tissue in the area considered to be the pith by the advocates of the receptacular theory (see Figure 18, Blaser and Einset[2]) is in part diploid and in part tetraploid. On the contrary tetraploidy in the ovary section, shown in Figure 14*A*, is mostly in the region considered to be "cortical" as in the stems. If the fruit was receptacular in origin, then tetraploidy should be more toward and between the region of "carpels," and not largely in the region of "cortex"; and no break in the continuity of tetraploid tissue should have occurred. The pattern of tetraploid tissue in most of the fruits of *2-2-4* chimeral Winesap would make the fruits resemble a leaf and, therefore, it may follow that the apple fruit is of appendicular—or foliar?—rather than of receptacular origin.

Blaser and Einset[2] show by a diagram (their Figure 8) that in the *2-2-4* chimeral fruit of Northern Spy there is *2x* tissue between adjacent tetraploid tissues. In their summary they state that "In 'Giant' Rome, with three layers of diploid initials, the floral tube contains cords of tetraploid cells enclosed in diploid tissue. This condition supports the view that the apple flower is composed of fused parts." It may be pertinent to quote in this connection Coulter *et al.*[3] (p. 255) who have stated: "All such terms as 'united,' 'fused,' 'coalescent,' and their technical equivalents suggest a very wrong impression as to the origin of the structure concerned. The separate parts implied in the words 'united,' etc., never had a separate existence. For example, in many cases separate petals occur; and where there is zonal development they are not separate, but this does not mean that they have 'united' or 'coalesced.'"

The situation in the fruit where fusion of parts is implied may be compared with an analogous situation which exists among pinnate, pinnatifid and entire leaves. In pinnate leaves the pinnae are developed because of lack of or restricted growth at the areas between the pinnae, whereas in the pinnatifid leaves the growth at comparable regions is less restricted and in the entire leaf such restriction of growth does not exist at all. In this case it would be incorrect to say that the entire leaf is derived from the fusion of pinnae. Similarly it should be understood that no true ontogenetic fusion of flower parts occurs in the development of the apple fruit. If we accept the premise that in the fruit there had been an ontogenetic fusion, for example, between a carpellary system and the flower tube, as indicated by Blaser and Einset (quoted above), then the *chimeral makeup* in the fruits had to be really more complex than has been observed. In such a case, the ploidy condition in the fruit of the *2-2-4* chimeral plant would have appeared, beginning from the outer epidermis to the inner epidermis, as: diploid-tetraploid-diploid-tetraploid-diploid. And besides, if it is assumed that the outer portion of the fruit is derived from fusion between the stalks of five sepals or petals or both, then there has to be five or a multiple of five separate tetraploid regions surrounded by diploid tissue. Such an implication is suggested by Blaser and Einset[2] (see their Figure 8), as they have stated: "Since the tetraploid tissue of each carpel is surrounded by diploid tissue. . ." Such a condition, as implied by Blaser and Einset, may be possible if from the beginning of flower initiation the flower parts were developed separately and later they came together and

formed a tight union and gave rise to the ovary (as defined earlier in the text). No such developments were observed in the chimeral fruits studied here (see Figures 14*A* and 17*B*).

Histological development in the ovary of apple flower may be compared with the histological development in the ovary of peach flower before part of the tissue in the ovary of peach flower becomes differentiated into the stony pit. The pit tissue in the stony fruits does not develop as an independent body of tissue at the early stages during the development of the pistil, but is developed merely by cellular differentiation into stony cells at later stages of ovary growth, normally following fertilization. It would seem it is exactly in the same manner that in the fruit of the apple, part of the tissue toward the ovarian cavity becomes somewhat differentiated; hence a "core line" between the differentiated tissue and the tissue toward the outer epidermis in the apple fruit. Furthermore, inside the fleshy part of the apple flower, the ovary, there is no region where a particular development of areas into "carpels" can be recognized. Regions in the mature fruit, generally designated as representing the carpels, become more or less apparent following certain physio-histological changes in the body of the fruit during the period of maturation. Again, judging from the *present* cytochimeral material, such areas in the body of the fruit do not seem to have any morphogenetic significance. It would seem that, since the pit in the stone fruit does not represent carpel, similarly in the apple no portion of the fruit inside the "core line" may be considered to represent carpels.

Perhaps then, on the basis of evidence presented in the text, it may be suggested that in the apple fruit, no region should be designated as being the "carpel" or "carpels" as separate identities or regions from the rest of the body of the fruit; the whole *fruit* itself may be designated to represent the *ovary* or *the-one-carpel*. In this case, the word "carpel" would really mean the fruit—*as the Greeks have it*—and not a body or bodies—five carpels—in the fruit, divided by imaginary lines from the rest of the fruit, in the sense as is now generally understood. It should again be pointed out here that the above interpretation is based strictly on the histogenetic development as observed in cytochimeral material. By this concept it is not meant to deny that phylogenetically the apple flower and the fruit represent compound structures which are derived from flowers with well differentiated parts of some ancestral plants. The phylogenetic theory has been amply supported by numerous studies of vascular anatomy[12]. Phylogeny of apple fruit based on anatomical structures refers to its past history; morphogenesis described on the cytochimeral basis reveals the development in the apple fruit as it is.

The present study and that of Blaser and Einset have dealt with quite similar problems, although the varieties of apples used as material in these studies differed. In their study tetraploidy follows rather faithfully the development of the vascular system in the leaf, as well as in the flower. It seems inconceivable that the differences between the present study and theirs on the flower can have resulted from environmental factors, since the same fundamental difference was recorded between the two studies made on the leaf: in this case both studies having been made on leaves of the same variety, Northern Spy[1,8]. Therefore, it would appear that the differences recorded herein were due to differences in the methods of observation. Blaser and Einset traced tetraploidy and diploidy of tissues by chromosome plates alone, whereas in the present study such determination was based on three criteria of ploidy combined: chromosome plate, nuclear size, and cell size. Chromosome plates alone cannot be sufficient to determine the boundaries between tissues with different ploidy, since chromosome plates are present in a very small percentage of cells in meristematic tissue. Boundaries of diploid and tetraploid tissues may be determined quite accurately by taking into consideration the total picture presented by chromosome plates, nuclear size, and cell size, especially when the areas studied are adjacent, belong to the same tissue, and appear in the same section. In the light of evidence here recorded, it seems that the diagrammatic sketches shown by Blaser and Einset represent the subject matter in a rather idealized perspective and that in most cases the areas occupied by diploid and tetraploid tissues are actually asymmetric rather than symmetrical as their sketches indicate.

Summary

The ontogenetic relationship between various tissues or regions in all the flower parts and the apical cell layers of the shoot of a 2-2-4 (diploid-diploid-tetraploid) chimeral Winesap was studied.

Ploidy determination in any tissue or in adjacent areas of a particular tissue or tissue system in all flower parts was based on three criteria: chromosome plates, nuclear size, and cell size. The localization of tissues of different ploidy is discussed in detail and presented in photomicrographs.

In some parts of some fruits of cytochimeral plants, tetraploidy was absent, whereas in the stem the pith was always tetraploid, even in chimeral plants of the 2-2-2-4 and 2-2-2-2-4 types. This indicates the resemblance of fruit to leaf; since no pith is present in either structure. This fact would appear to support the theory of *appendicular* origin of the *fruit* of the apple.

The pattern of tetraploid area in the fruit resembled that in the leaf; in both it was mostly asymmetrical. Based on this similarity, it was concluded that the apple fruit does not represent fusion of flower parts in the strict sense. Fusion of parts in the flower would have to be understood only in a phylogenetic sense. Based entirely on cytohistological evidence, it is suggested that the lower, fleshy part of the apple flower may be considered as being a true ovary which matures into the fruit. No "carpels," as generally defined, could be recognized in the apple fruit of the chimeral plant.

Literature Cited

1. Blaser, H. W. and J. Einset. Leaf development in six periclinal chromosomal chimeras of apple varieties. *Amer. Jour. Bot.* 35:473-482. 1948.
2. ———— and ————. Flower structure in periclinal chimeras of apple. *Amer. Jour. Bot.* 37:297-304. 1950.
3. Coulter, J.M., C. R. Barnes and H. C. Cowles. Textbook of Botany, Vol. 1. American Book Company. New York. 1910.
4. Dermen, Haig. The mechanism of colchicine-induced cytohistological changes in cranberry. *Amer. Jour. Bot.* 32:387-394. 1945.
5. ————. Periclinal cytochimeras and histogenesis in cranberry. *Amer. Jour. Bot.* 34:32-34. 1947.
6. ————. Chimeral apple sports and their propagation through adventitious buds. *Jour. Hered.* 39:235-242. 1948.
7. ————. Tetraploid and diploid adventitious shoots from a giant sport of McIntosh apple. *Jour. Hered.* 42:144-149. 1951.
8. ————. Ontogeny of tissues in stem and leaf of cytochimeral apples. *Amer. Jour. Bot.* 38:753-760. 1951.
9. Gourley, J. H., and F. S. Howlett. Modern Fruit Production. The Macmillan Company. New York. 1941.
10. Kraus, E. J. The pollination of pomaceous fruits. I. Gross morphology of the apple. *Oregon Agric. Exp. Sta. Res. Bull.* 1:1-12. 1913.
11. ———— and G. S. Ralston. The pollination of pomaceous fruits. III. Gross vascular anatomy of the apple. *Oregon Agric. Exp. Sta. Bull.* 138:1-12. 1916.
12. MacDaniels, L. H. The morphology of the apple and other pome fruits. *Cornell Univ. Agric. Exp. Sta. Mem.* 230. 1940.
13. Payer, J. B. Traité D'organogénie Comparée de la Fleur. Paris, France. 1857.
14. Satina, Sophie. Periclinal chimera in *Datura* in relation to the development and structure (a) of the style and stigma (b), of calyx and corolla. *Amer. Jour. Bot.* 31:493-502. 1944.
15. ————. Periclinal chimeras in relation to the development and structure of the ovule. *Amer. Jour. Bot.* 32:72-81. 1945.
16. ———— and A. F. Blakeslee. Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. *Amer. Jour. Bot.* 28:862-871. 1941.
17. ———— and ————. Periclinal chimeras in *Datura* in relation to the development of the carpel. *Amer. Jour. Bot.* 30: 453-462. 1943.
18. Tukey, H. B. and J. O. Young. Gross morphology and histology of developing fruit of the apple. *Bot. Gaz.* 104: 3-25. 1942.

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A Direct Proof of the Relation between Genotypic Mating Correlation and Gametic Uniting Correlation in Equilibrium Populations

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LET m be the ((product-moment) correlation coefficient between mating individuals in a population and F be that for uniting gametes. It has been shown[2] that in all equilibrium populations, $F = \frac{m}{2-m}$ (1) whatever the gene frequencies as long as the gene effects are additive. The demonstration of (1) was based on two path coefficients—one for the path from uniting gametes to the resulting zygote and another from a zygote to gametes produced by it. This note is to give a direct proof of the relation (1) without introducing the path coefficients.

Let Z take the values 2, 1, 0, corresponding to genotypes AA, Aa, aa, respectively, whose proportions in an equilibrium population are x, $2y$, z, where $x + 2y + z = 1$. Further, let u_{22} be the frequency of the mating $AA \times AA$ among all the matings in the population, and assume reciprocal matings to be equally numerous so that $u_{21} = u_{12}$ and $u_{21} + u_{11} = 2u_{21}$.

TABLE 1. Frequencies of Genotypic Matings

	AA (2)	Aa (1)	aa (0)	
AA (2)	u_{22}	u_{21}	u_{20}	x
Aa (1)	u_{12}	u_{11}	u_{10}	$2y$
aa (0)	u_{02}	u_{01}	u_{00}	z
	x	$2y$	z	1

TABLE II. Gametic Correlation

	A (1)	a (0)	
A (1)	x	y	$x+y$
a (0)	y	z	$y+z$
	$x+y$	$y+z$	1

The frequencies of genotypic matings and the gametic correlation table are given in Tables I and II respectively.

Before proceeding with the demonstration, we need to establish a preliminary theorem which is true for all equilibrium populations. From Table I it is seen that the genotypic proportions are:

for parents: $x = u_{22} + u_{21} + u_{20}$, etc.

for offspring:

$x = u_{22} + \frac{1}{2}(u_{21} + u_{12}) + \frac{1}{4}u_{11}$, etc.

Since the population is in equilibrium, these two expressions must be equal. But, $\frac{1}{2}(u_{21} + u_{12}) = u_{21}$; hence (Haldane and Moshinsky[1]),

$$u_{11} = 4u_{20} = 2(u_{20} + u_{02}). \qquad (2)$$

That is to say, in all equilibrium populations, the frequency of the mating $Aa \times Aa$ is twice that of $AA \times aa$, reciprocals included.

Now, we may calculate the value of m from Table I. The mean value of Z is $2(x+y)$, and its variance is

$$\sigma_z^2 = 4x + 2y - 4(x+y)^2 = 4(xz-y^2) + 2y.$$

The covariance between the mating genotypes is

$$\sigma_{zs} = 4u_{22} + 4u_{21} + u_{11} - 4(x+y)^2$$

by (2),

$$= 4x - 4(x+y)^2 = 4(xz - y^2).$$

$$\therefore m = \frac{4(xz-y^2)}{4(xz-y^2)+2y} \qquad (3)$$

TABLE III. Frequencies of Matings at Equilibrium

	AA	Aa	aa	
AA	$(1-\omega)x^2+\omega x$	$2(1-\omega)xy$	$(1-\omega)xz$	x
Aa	$2(1-\omega)xy$	$(1-\omega)4y^2+2\omega y$	$2(1-\omega)yz$	$2y$
aa	$(1-\omega)xz$	$2(1-\omega)yz$	$(1-\omega)z^2+\omega z$	z
	x	$2y$	z	1

and $2-m = \dfrac{4(xz-y^2)+4y}{4(xz-y^2)+2y}$

Hence $\dfrac{m}{2-m} = \dfrac{xz-y^2}{xz-y^2+y}$ (4)

On the other hand, the value of F may be directly calculated from Table II which yileds

$$F = \frac{xz-y^2}{(x+y)(y+z)} \qquad (5)$$

Since $(x+y)(y+z) = xz + xy + y^2 + yz = xz + y(1-y) = xz - y^2 + y$, (5) is equal to (4). Hence, (1) is proved. In a random mating population, $xz - y^2 = 0$, and $m = F = O$. That the correlations between parent and offspring and between sibs are both $\frac{1}{2}(1+m)$ may be demonstrated in a similar manner.

A Special Case

If a fraction ω of the individuals in a population practices self-fertilization in every generation while the rest mate at random, the population will soon reach an equilibrium condition. The frequencies of the various matings at equilibrium are given in Table III.

It may be readily verified by direct calculation that the correlation coefficient between mates of Table III is $m = \omega$. But a much shorter procedure is to use the relation (2) by which we have

$$4(1-\omega)y^2 + 2\omega y = 4(1-\omega)xz;$$

$$\therefore \omega = \frac{2(xz-y^2)}{2(xz-y^2)+y} = m \qquad (6)$$

The result that $F = \omega/(2-\omega)$ in an equilibrium population with ω self-fertilization has been arrived at by various authors previously but the demonstration given here in terms of m emphasizes the fact that it is a particular case of the general theorem (1).

Literature Cited

1. Haldane, J. B. S. and P. Moshinsky. Inbreeding in Mendelian populations with special reference to human cousin marriage. *Ann. Eugen.* 9:321-340. 1939.

2. Wright, Sewall. Systems of mating. I. The biometric relations between parent and offspring. *Genetics* 6:111-123. 1921.

TWO NON-ALLELIC MUTANTS RESEMBLING "W" IN THE HOUSE MOUSE

LEONELL C. STRONG AND W. F. HOLLANDER*

THE "dominant white" coloration in mice has long been of interest since the homozygous condition has severe anemia which is ordinarily lethal within a few days after birth. More recently a second mutant allele was discovered by Little and Cloudman which produces only mild anemia when homozygous. These two alleles have been symbolized W and W^v respectively. A review of studies concerning them was given by Grüneberg[2]; and later studies of significance were made by Attfield[1] and by Russell.[3]

Grüneberg has pointed out that the phenotypes exhibited by $W//+$ mice are markedly varied, due to modifiers. Ordinarily a moderate amount of white spotting is shown, together with some greying or "roaning." With $s//s$ (recessive piebald spotting) the phenotype is, as a rule, mainly white, with black eyes. $W^v//+$ on the other hand ordinarily produces less variegation, but more dilution in the pelage color.

Strong and Hollander[4] described a mutant which resembled W in general, and which appeared in an inbred strain (N) treated with methylcholanthrene. Subsequently another somewhat similar mutant appeared in a methylcholanthrene. treated mouse of another strain. In the present paper a study of these two mutants is reported.

History of Origins

None of the inbred strains at this laboratory carried W originally, and the gene has never been introduced. Furthermore, W-like mutants have occurred only in stocks subjected to subcutaneous injection of methylcholanthrene dissolved in sesame oil, for a number of generations.

The first mutant was discovered in a mouse of the N-strain in 1945 (type 1). Mice of this strain are ordinarily piebald (and dilute; non-agouti; brown). The mutant showed considerably lighter pigmentation and more extensive white areas. Many crosses and breeding tests showed that the essential mutant here was of the W type, the homozygote being anemic and dying young. In general, however, the heterozygotes are more like W^v in phenotype.

The second mutant appeared in 1949, in a strain called 3CAMG. The history of this strain is of interest here. Originally a cross of three strains, CBA, N and JK had been made, the composite being called NHO. Subsequently brother-sister mating was consistently practiced, and beginning with F_4 most derivative lines were treated with methylcholanthrene. One of these was Br-S, of self non-agouti brown color (Strong and Hollander[4]). From this strain the 3CAMG sub-line was isolated. It differed in three features: A small frontal blaze, a white belly streak, and somewhat light-colored frontal pelage. It is possible that the white markings were derived from the N-strain—the only ancestor showing white. At any rate, the 3CAMG type bred true. Crosses with ordinarily piebald mice produced self hybrids.

The W-like mutant which appeared in this strain (type 2) showed a large frontal blaze, a large patch on the belly, and several white spots on the shoulders and back. With ordinary 3CAMG mates the mutant produced equal numbers of

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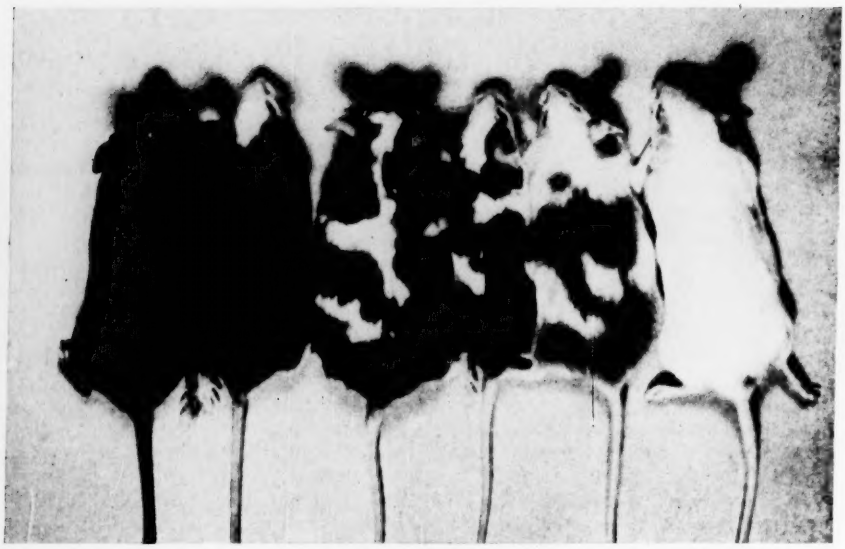

SIX SPOTTING PHENOTYPES

Figure 19

All these mice are non-agouti black. Shown from left to right are: Frontal star or small blaze, probably one of the "K complex," modifiers of piebald spotting; Mutant dominant spotting type 1, associated with blaze, (note increased size of blaze and lightened peage generally); Extreme example of dominant spotting type 2, associated with blaze, (note resemblance of phenotype to the following one, and absence of lightening of pelage); Ordinary piebald *c/ / s;* Combination of piebald and dominant spotting type 1; Combination of piebald and dominant spotting type 2.

offspring of both types, classification being quite easy.

Further Genetic Tests

Matings of the mice of mutant type 2 with each other (in the strain) produced 1430 classifiable offspring, which fell into only two classes. The ratio was 930 of the mutant type : 500 of the 3CAMG type, or less than 2:1 (actual 34.96 percent self—expected 33.33 percent self or 1.63 ± 1.79 or 0.91 × P.E.). The difference from a 3:1 ratio is very significant (actual 34.96 percent self—expected 25.00 percent self or 9.96 ± 1.65 or 6.04 × P.E.). Evidently the homozygous dominant class was lethal, and since no lethality was observed after birth, an examination of fetuses from this type of mating was made. Anemic fetuses were recognizable at about the 15th day of pregnancy, and many of these were aready dying.

Outcrosses of the type 2 mutants to self stocks yielded F_1 hybrids of two main classes; self or with some white. However, the amount of white varied in the latter from merely a large belly spot to somewhat variegated with blaze. On the other hand, outcrossing to ordinary piebald stocks gave a clearer division; about half the young appeared to be piebald, and the rest were self or had a small blaze. Backcrosses of the piebald F_1 to the piebald parent type produced a great variety of offspring, but about a fourth of them were almost or entirely white, with pigmented eyes.

Matings *inter se* of mongrels having the type 2 mutant again gave a 2:1 segregation, but a very few anemic young survived to be born. The detailed data are given in Table I.

Since neither of the present mutants conformed closely with the previously known *W* or W^v, further tests seemed desirable. The question as to whether these new mutants were alleles of one another was tested by crossing them reciprocally. It was expected that the presence of both mutants in one zygote would be anemic if they were alleles. To date, 219 F_1 offspring have been classified as to color. Four classes were found: 48 of type 1; 64 type 2; 65 self or with small blaze only and 42 with piebaldness. This last class was apparently the combination phenotype, and it was clearly not anemic nor even sterile. Furthermore, examination of late pregnancies in this cross showed no anemic or late-dying class at all (Table I).

The four classes of hybrids in the latter cross were further tested among themselves (Table II) with results confirming the idea that the mutants are not allelic. Finally several of the hybrids supposedly carrying both mutants together were outcrossed to C57 mice (self black). These produced 44 young as follows: 12 self, 13 with pale ventral pelage and white belly spot, 2 with pale ventrum only, and 17 with white spots dorsally and ventrally. This is a repulsion-phase linkage test and if the two mutants were allelic or pseudo-allelic, no self-colored offspring would be expected. The phenotypes of the other classes are probably confused and it seems likely that C57 mice have darkening modifiers for the type 1 mutant, especially regarding the dorsal pelage. This test therefore needs repetition with other strains.

The question remaining is whether either of the present mutants is allelic with the previously known *W*. Through the kindness of Dr. Borges, *W* mice of one of Dr. Russell's stocks (having white ventral patch) were obtained, and crossed with our mutant type 2. In the F_1, 67 mice have been produced (48 with white spotting : 18 self). No anemic young were observed, but the phenotypes of the F_1, and the closeness to a 2:1 ratio indicate that there is intra-uterine lethality.

Discussion

While the analysis of several interactions and interrelations remains to be completed, it is already clear that the *W* locus is not unique in showing a relation between pelage color and anemia. A fundamental connection between melanin and hemoglobin production seems indicated.

Grüneberg[2] has suggested (p. 150) that the *W* locus is unstable, since mutations of it have occurred several times. We are not inclined to agree with Grüneberg on this point, as no such mutation has appeared in scores of thousands of self-colored mice in this laboratory. It is noteworthy that both the present mutants appeared in strains treated with methylcholanthrene, which has a mutagenic effect.

Another interesting point is that both the present mutants appeared in stocks already possessing some white. It would be of considerable interest to discover whether a step series of mutations is involved. As yet the difficulties of classification in many crosses involving white spotting, mainly because of the numerous modifying genes, such as the "K complex" and the "m (*W*) complex," have deterred us from such an analysis.

Summary

Two mutants resembling *W* (dominant white spotting, lethal anemic homozygous) are described and their origins outlined. Breeding tests show some differences in each case from *W*, with color phenotype or time of lethality. One is apparently allelic with *W*, the other

TABLE II. Coat-color phenotypes in F_2 from cross of mutant type 1 × type 2

F_1 color types and ratio expected in progeny	self	type 1	F_2 type 2	Piebald (both)
1 × 2 (1:1:1:1)	31	29	40	27
1 × 1 (1 : 2)	32	56	0	0
2 × 2 (2 : 1)	13	0	21	0
Piebald (both) × Piebald (both)	6	20	17	28

TABLE I. Fetal counts in matings involving type 2

Parental type Sire	Dam	No. preg.	Total young	Ave. litter	Normal young	Early* dead resorbing	Anemic or late dead	% Anemic	σ
2 Inbred	2 Inbred	21	185	8.8	141	14	30	16.2	± 2.7
2 Mongrel	2 Mongrel	29	257	8.9	211	26	49	19.1	± 2.4
Combined above gestation days or over		15	24	245	10.2	176	20	49	20.0	± 2.5
1 (15 + days)	Inbred 2 Mongrel	9	81	9.0	75	6	—	—	
1 (15 days +)	2 Mongrel	3	35	11.5	34	0	1 (dead)	—	
2 Mongrel	1	14	143	10.2	127	13	3 (dead)	12	

*Early dead embryos are not uncommon in all stocks, with *W* or not. Late deaths (after 15th day) are rare.

σ = standard error of % $= \sqrt{\frac{(\%)\,(100 - \%)}{n}}$

Example: 245 young, of which 20% are anemic

$= \sqrt{\frac{(20)\,(80)}{245}} = 2.5$

not. The compound of both mutants is viable and fertile.

Literature Cited

1. ATTFIELD, M. Inherited macrocytic anaemias in the house mouse. III. Red blood cell diameters. *Jour. Genet.* 50:250-263. 1951.

2. GRÜNEBERG, H. The Genetics of the Mouse. Cambridge University Press. 1943. 2nd. Ed. Martinus Nijhoff, The Hague. 1952.

3. RUSSELL, E. S. Analysis of pleiotropism at the W-locus in the mouse. *Genetics* 34:708-723. 1949.

4. STRONG, L. C., and W. F. HOLLANDER. Spontaneous gastric neoplasia in mice of the Br.-S strain: incidence and linkage tests. *Cancer Research* 11: 94-99.

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GENETICS CONGRESS TRAVEL GRANTS ANNOUNCED

THE Travel Committee of the Genetics Society of America has succeeded in raising a sum of money to assist members to attend the Ninth International Congress of Genetics which will be held in Bellagio, Italy, August 24-31, 1953. The organizations that have so generously contributed to this fund are:

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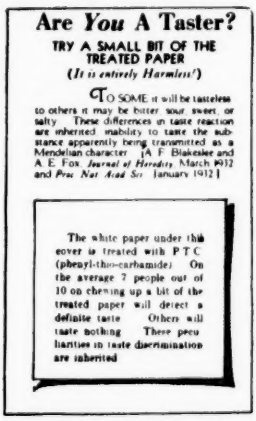
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